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㉙ Articles having bioactive surfaces.

㉚ The invention relates to articles having bioactive agents coupled to or repelled by a graft polymer which is in turn bonded to a polymeric substrate surface. The articles are useful as thromboresistant and/or antimicrobial medical devices.

The invention relates to articles (hereinafter "blood contact articles") which in use contact blood or blood products, and in particular such articles having a polymeric working (contact) surface.

For over forty years a number of medical devices which contact the blood or blood products of living persons or animals have been developed, manufactured and used clinically. Examples of such articles include pacemakers, arterial grafts, heart valves, artificial hearts, heart pumps, hip prostheses, heart lung machines, catheters and kidney dialysis equipment.

A major problem with such articles is that their working (blood contact) surfaces (i.e. surfaces which contact blood or blood products, including for example serum, plasma and other fluids and solids derived from blood) are foreign to blood and blood products and tend to initiate, among other things, red cell destruction and coagulation of blood to form clots (thrombogenesis).

Normal intact endothelium is nonthrombogenic due partly to the synthesis of heparan sulfate. Heparan sulfate tends to remain bound to the surface of endothelial cells accelerating the inactivation of thrombin, the enzyme responsible for the polymerization of fibrinogen to fibrin in clot formation, by antithrombin III (ATIII). Heparan sulfate is a very powerful anticoagulant in the natural vasculature. Consequently, it has been of great interest to physicians and the medical industry to devise blood-contacting polymeric surfaces that possess characteristics of heparan sulfate, specifically by coating surfaces with heparin. For example, in US-A-3826678 (Hoffman et al.) biologically active molecules are chemically bonded to polymers and copolymers which previously have been radiation-grafted to inert polymeric substrates such as polyurethane and polyethylene. The grafted polymer is preferably a hydrophilic hydrogel (e.g. a hydroxyethylmethacrylate (HEMA) polymer) and may include heparin bonded to the hydrogel.

Another major problem with such articles is their susceptibility to post-implant infection. Staph. epidermidis, which exists on human skin and Staph. aureus, sometimes found in hospital environments, are the two most frequent pathogens encountered in implant and similar situations. They both have the ability to enter the body through the surgical opening and attack the implant site. This problem has been addressed, for example, in US-A-4442133 (Greco et al.) in which a PTFE or Dacron graft is soaked in TDMAC (tridodecyl methyl ammonium chloride) dissolved in ethanol. The TDMAC is absorbed to form a coating and then incubated in a solution of antibiotic, eg. penicillin or cefoxitin. Also, for example, in US-A-4612337 (Fox, Jr., et al.) a polymeric material such as PTFE is soaked in antibiotic solutions with an organic solvent, then soaked in an organic solvent with a metal salt followed by a resoak in the antibiotic/organic solvent solution.

There is therefore a clear need for new and improved thromboresistant and infection resistant articles.

It has now been found that articles which in use contact blood or blood products have improved properties in this regard where they have a working surface comprising a polymeric surface with grafted thereon a selected vinyl graft copolymer with coupled thereto a bioactive agent.

Viewed from one aspect the invention thus provides an article for use in contact with blood or blood products, said article having at least one exposed surface provided by a polymeric substrate with grafted thereto a graft polymer deriving from a hydrophilic vinyl monomer and with coupled to said graft polymer a bioactive agent, said graft polymer where non-ionically coupled to a said bioactive agent being a polymer or copolymer of a vinyl monomer selected from acrylic acid (AA), acrylamide (AAm), N-(3-aminopropyl)-methacrylamide (APMA), 2-acrylamido-2-methylpropanesulfonic acid (AMPS) and salts thereof.

In the articles of the invention the bioactive agent can conveniently be ionically, covalently or physically coupled to the graft polymer and can be an antibiotic or antimicrobial, such as silver or gentamycin or other conventional infection combatting agents, or an anticoagulant or other bioactive agent combatting thrombogenesis. The polymeric substrate surface of the article onto which the graft polymer is grafted is conveniently a polyurethane such as a polyetherurethane. The advantages provided by such an article involve features of both hydrophilic coatings and bioactive agents. For example, antimicrobials are able to fight post-implant infections while hydrophilic coatings such as an AMPS-containing coatings are able to reduce thrombosis and provide slippery surfaces.

In one embodiment of the blood contact article of the invention, the graft polymer includes pendant groups having an ionic charge and the bioactive agent is ionically coupled thereto in that it has an ionic charge of opposite polarity to that of the graft polymer's pendant groups. For example, the graft polymer could be AMPS or APMA and the bioactive agent could be gentamycin or basic fibroblast growth factor (bFGF). The bioactive agent is thereby coupled to and maintained on the article by ionic attraction.

In another embodiment of the blood contact article of the invention, the bioactive agent has an ionic charge of the same polarity as that of the pendant groups of the graft polymer. With this embodiment, in use the bioactive agent is therefore repelled and released from the surface of the article into the blood.

In yet another embodiment of the blood contact article of the invention, an albumin-binding dye such as blue dextran may be used as the bioactive agent and can be ionically or covalently attached to the graft polymer, e.g. an APMA/AAm copolymer. The surface then attracts albumin from the blood contacting the surface of the

article.

In a yet further embodiment of the blood contact article of the invention, the graft polymer may be used to provide control of hydrophilicity and coupling of the bioactive agent by appropriate selection of the monomers for the graft polymer from AAm, AA, AMPS, HEMA and APMA in predetermined relative amounts. For example, a copolymer of APMA and AAm can be grafted onto a polyurethane surface and heparin can be coupled to the grafted copolymer.

The invention allows simultaneous solution of a number of problems encountered with implantable articles. The invention involves features of hydrophilic coatings and bioactive agents, such as antimicrobials, which are able to fight post-implant infections as well as thrombosis while providing slippery surfaces. Other bioactive agents are contemplated as well.

Thus the invention meets the need for new and improved thromboresistant/infection-resistant blood contact articles, eg. by providing such articles with antimicrobial agents which can result in bactericidal activity of that polymer, or with anticoagulant agents to impart thromboresistance.

As indicated above however it is also sometimes desirable to include bioactive agents other than antimicrobials and anticoagulants in such articles.

The substrate of a working surface of an article intended for contact with blood or blood product will generally be comprised of a biologically inert polymeric material. A hydrophilic graft polymer having a net charge due to pendant anionic or cationic groups thereon may be permanently covalently bonded to the polymeric substrate by graft polymerization. Such graft polymers can be selected to provide thromboresistant slip coatings which lend themselves to ionic coupling (when selected to provide an appropriate charge dissimilar to that of the bioactive agent) with various ionic bioactive agents. Ionic coupling of the bioactive agent to the graft polymer may be achieved by simply immersing the surface-grafted polymer in a solution of the desired bioactive agent. Other graft polymers can be selected to provide hydrophilic slip coatings which lend themselves to covalent coupling (when selected to provide a functional chemical group appropriate for the covalent attachment of the bioactive agent) with bioactive agents.

The various articles contemplated by this invention may for instance have solid-phase polymeric substrates selected from the group of materials shown in Table 1 below.

TABLE 1

Polyamides
Polycarbonates
Polyethers
Polyesters
Polyolefins
Polystyrene
Polyurethane
Polyvinyl chlorides
Silicones
Polyethylenes
Polypropylenes
Polyisoprenes
Polytetrafluoroethylenes

At the present time it is believed that polyurethane provides the preferred polymeric substrate in the context of this invention.

The graft polymer to be used conveniently originates with a grafting monomer which is hydrophilic. Such monomers create a hydrophilic polymer coating on the substrate surface. A hydrophilic coating minimizes protein interactions and also provides slip properties to the surface. The monomer must contain at least one vinyl group. The vinyl group is necessary for free radical polymerization to occur. The monomer may be neutral,

anionic or cationic. After grafting (and if necessary following chemical modification) the resultant grafted polymer should have a net positive or net negative charge. The charge allows the ionic attachment of oppositely charged bioactive molecules. Neutral monomers may be chemically modified after grafting to yield anionic or cationic surfaces. For example, grafted acrylamide may be hydrolyzed to acrylic acid thus forming an anionic surface.

Specifically, a number of graft slip coatings have been used according to this invention. The most preferred are comprised of monomers grafted onto the substrate surface via ceric ion initiation. Monomers with cationic as well as anionic pendant groups have been grafted. An example of the former is N - (3 - aminopropyl) methacrylate (APMA) and copolymers thereof while a prime example of the latter is 2- acrylamido -2-methyl propane sulfonic acid (AMPS).

These charged surfaces lend themselves to the ionic coupling of charged bioactive agents. The ionic coupling of the bioactive agents has the advantage of slowly releasing these agents under appropriate conditions. Examples of antimicrobial bioactive agents are gentamycin sulfate and silver ion. Generally, bioactive agents selected from the types shown in Table 2 may be used according to this invention for ionic or covalent coupling.

TABLE 2

	Antibacterial agents or antimicrobial agents
	Anticoagulants
	Enzymes
	Catalysts
	Hormones
	Growth factors
	Lectins Drugs
	Vitamins
	Antibodies
	Antigens
	Nucleic acids
	Dyes - which act as biological ligands
	DNA and RNA segments
	Proteins
	Peptides

Thus viewed from a further aspect the invention provides a process for the provision of an implantable article with a bioactive surface, said process comprising:

graft polymerizing on an exposed polymeric surface of said article a hydrophilic vinyl monomer having pendant groups optionally carrying an ionic charge,

if required chemically modifying the resultant graft polymer surface to provide an ionically charged or ionizable surface, and

coupling to said graft polymer surface a bioactive agent.

In a preferred aspect of the process of the invention, an implantable article is provided with a bioactive surface by:

contacting an implantable article having an exposed polymeric surface with an aqueous solution of C IV ion and a vinyl monomer whereby to generate a graft polymer surface on said polymeric surface, and attaching a bioactive agent to said graft polymer surface. The aqueous monomer solution may contain one or more vinyl monomers and the implantable article may be contacted by different successive solutions whereby to build up layered or copolymeric graft polymer surfaces if so desired.

Thus one embodiment of the method comprises the steps of:

- (a) preparing an aqueous solution of AMPS and Ce IV ion;
- (b) contacting a polymeric surface of an implantable article with said aqueous solution for a period of time effective to provide a polymer of AMPS grafted to the surface; and
- (c) attaching a bioactive agent to the grafted surface.

In this embodiment, by way of example the attachment of the bioactive agent may be by ionic binding, eg. of a cationic antimicrobial compound such as gentamycin, kanamycin, neomycin, silver ion, chlorhexidine, vancomycin, streptomycin or erythromycin, or of an agent such as basic fibroblast growth factor or a cationic dye such as toluidine blue.

A further embodiment of the method comprises the steps of:

- (a) preparing an aqueous solution of AAm, APMA and Ce IV ion;
- (b) contacting a polymeric surface of an implantable article with said aqueous solution for a period of time effective to provide a polymer of the AAm and APMA grafted to the surface; and
- (c) attaching a bioactive agent to the grafted surface.

In this embodiment also by way of example the attachment of the bioactive agent may be by ionic binding, eg. of an anionic microbial compound such as ampicillin, oxacillin, cefazolin, bacitracin, cephalosporin, cephalothin, cefuroxime, cefoxitin, norfloxacin, perfloxacin or sulfadiazine, or of an anionic dye such as Ponceau S.

A yet further embodiment of the method comprises the steps of:

- (a) preparing an aqueous solution of AAm, APMA and Ce IV ion;
- (b) contacting a polymeric surface of an implantable article with said aqueous solution for a period of time effective to provide a polymer of AAm and APMA grafted to the surface; and
- (c) attaching a bioactive agent to the grafted surface.

In this embodiment, by way of example attachment of the bioactive agent may be by covalent bonding, eg. of an albumin-binding dye, such as Cibacron blue. In this event the covalent coupling of the bioactive agent may be effected by the steps of:

- (a) applying an oxidizing agent, such as a periodate, to a carbohydrate, such as dextran, to form aldehyde groups on the carbohydrate;
- (b) attaching the oxidized carbohydrate to the grafted surface by reductive amination;
- (c) treating the attached carbohydrate with an imine linkage stabilizing agent, such as sodium cyanoborohydride; and
- (d) covalently coupling the bioactive agent to the reduced carbohydrate.

A still further embodiment of the method of the invention comprises the steps of:

- (a) preparing an aqueous solution of (i) a first monomer selected from AAm, AA and AMPS (preferably AAm), (ii) a second monomer selected from HEMA and APMA (preferably APMA), and (iii) Ce IV ion;
- (b) contacting a polymeric surface of an implantable article with said aqueous solution for a period of time effective to provide a copolymer of said first and second monomers grafted to the surface; and
- (c) covalently attaching heparin to the grafted surface by
- (d) applying an oxidizing agent, eg. nitrous oxide or sodium periodate, to the heparin to form aldehyde groups on the heparin;
- (e) attaching the oxidized heparin to the grafted surface by reductive amination; and
- (f) treating the attached heparin with an imine linkage stabilizing agent such as sodium cyanoborohydride.

While ceric ion initiation (CeIV) is presently most preferred as the technique to be used to graft monomers to substrate surfaces, other grafting techniques are well known and may be used in appropriate situations. For example, corona discharge, UV irradiation and ionizing radiation (^{60}Co , X-rays, high energy electrons, plasma gas discharge) are known. These grafting techniques are examples of how to form free radicals on a polymer substrate working surface. The free radicals formed thereon initiate the grafting of vinyl ($\text{CH}_2=\text{CH-R}$) type monomers.

The ionic coupling of bioactive agents is achieved by simply immersing the surface-grafted polymer in a solution of the desired bioactive agent. For example, in the case of antimicrobials, bactericidal (ring of inhibition) testing was performed on AMPS/ AgNO_3 and AMPS/gentamycin sulfate surfaces. Significant antibacterial activity was achieved. In the case of the AMPS/gentamycin sulfate surface, full activity was retained even after a 24 hour DI water rinse.

Although the detailed discussion below mentions Examples in which treatment is on films as the polymeric substrate surface, it is not intended that this invention be so limited. Grafted hydrophilic surfaces ionically coupled with bioactive agents may be similarly bound to other substrate surfaces, i.e., surfaces of articles intended to contact blood or blood products, of articles of any shape or form including tubular, sheet, rod and articles of proper shape for use in artificial organs, blood handling equipment or bodily implants of any kind and to any encapsulant means therefor.

The provision of body contact articles with an ionically charged graft polymer surface, such as that in the blood contact articles of the invention, may also be used to prevent interaction between ionic bioactive agents administered to the body by selection of a graft polymer having a surface charge of opposite polarity to that of the active ion of the bioactive agent.

5 Thus, viewed from a still further aspect the invention provides a method

- (a) preparing an aqueous solution of AMPS and Ce IV ion;
- (b) contacting a polymeric surface of an implantable article with said aqueous solution for a period of time effective to provide a polymer of AMPS grafted to the surface; and
- (c) attaching a bioactive agent to the grafted surface.

10 In this way ionic attraction between the administered bioactive agent and the body contact article surface is prevented.

As already indicated, normal intact endothelium is nonthrombogenic due partly to the synthesis of heparan sulfate. This heparan sulfate tends to remain bound to the surface of endothelial cells, accelerating the inactivation of thrombin, the enzyme responsible for the polymerization of fibrinogen to fibrin in clot formation, by ATIII. Heparan sulfate is a very powerful anticoagulant in the natural vasculature.

15 Heparin is a strongly acidic glycosaminoglycan. It has a high content of N- and O- sulfate groups. Heparin is structurally similar to heparan sulfate although it is more sulfated. The anticoagulant activity of heparin is directly dependent on its molecular size and electric charge, thus increasing the molecular weight and/or the amount of sulfonation will increase the anticoagulant activity. Therefore, a highly sulfonated polymer surface may stimulate the inactivation of thrombin by ATIII, similar to heparin.

The 2-acrylamido-2-methylpropane sulfonic acid (AMPS) coating is aimed at producing a surface that will decrease the nonspecific adsorption of various proteins due to its hydrophilicity and provide a highly sulfonated surface that will preferentially adsorb ATIII.

25 Much of the AMPS work effort relative to this invention went into developing a general AMPS surface modification technique for polyurethanes, however, the technique may be used for other material surfaces with few modifications. The technique described in detail below is based on the generation of free radicals on a polyurethane surface with CeIV ion and the graft copolymerization of AMPS monomers directly to that surface.

The process and articles of the invention will now be described further by way of illustration with reference to the following non-limiting Examples and to the accompanying drawings, in which:-

30 Fig. 1 is a graph showing the amount of toluidine blue dye released from the surfaces of Pellethane 55D polyurethane and Pellethane 55D polyurethane grafted with AMPS;

Fig. 2 is a graph showing a comparison of ATIII activity of uncoated Pellethane 55D polyurethane, heparin-coated Pellethane 55D polyurethane and AMPS-coated Pellethane 55D polyurethane samples (the results are expressed as the amount of thrombin inactivated by the sample surfaces/cm²);

35 Fig. 3 is a graph showing ring of inhibition of Pellethane 55D polyurethane, AMPS-coated Pellethane 55D polyurethane and AMPS-coated Pellethane 55D polyurethanes containing adsorbed gentamycin (the bacteria used were *S. epidermidis*);

Fig. 4 is a graph showing elution rate of gentamycin from AMPS-grafted Pellethane 55D polyurethane samples in deionized (DI) water, 0.9% NaCl solution and 10% NaCl solution as measured by ring of inhibition;

40 Fig. 5 is a graph showing ATIII activity of AMPS-coated Pellethane 55D polyurethane containing gentamycin and AMPS-coated Pellethane 55D polyurethane after 24 hour elution of gentamycin;

Fig. 6 is a graph showing the amount of fibroblast growth factor which binds to polyurethane (PU), and to polyurethane grafted with acrylamide (AAm), N-(3-aminopropyl) methacrylamide hydrochloride (APMA) and acrylamide (AAm) copolymer or 2-acrylamido-2-methylpropane sulfonic acid (AMPS);

45 Fig. 7 is a graph showing toluidine blue dye adsorption for the same substrates as in Figure 6 (i.e. PU, AAm, APMA/AAm and AMPS);

Fig. 8 is a graph showing the binding of Ponceau S dye to the same substrates as in Figure 6 and Figure 7 (i.e. PU, AAm, APMA/AAm and AMPS);

50 Fig. 9a shows a postulated reactive mechanism for CeIV ion initiation of free radicals on a polyurethane surface;

Fig. 9b shows the graft polymerization of AAm and APMA on a CeIV ion activated polyurethane surface;

Fig. 10 shows the periodat oxidation of dextran;

Fig. 11 shows the reductive amination (Shiff base reaction) of oxidized dextran and grafted APMA;

Fig. 12 shows the sodium cyanoborohydride stabilization of imine linkage;

55 Fig. 13 shows covalently coupled Cibacron Blue dye to surface bound dextran;

Fig. 14 is a graph showing ¹²⁵I-Albumin adsorption to Pellethane 55D polyurethane, Blue Dextran bulk derivatized Pellethane 55D polyurethane (55D/BD) and Blue Dextran surface derivatized Pellethane 55D polyurethane (Blue Dextran);

Fig. 15 is a graph showing ^{125}I -Albumin adsorption to uncoated Pellethane 55D polyurethane, APMA/AAM surface-coated Pellethane 55D polyurethane, Dextran surface-coated 55D polyurethane and Blue Dextran surface-coated Pellethane 55D polyurethane;

Fig. 16 is a graph showing Immunogold assay of albumin adsorbed to APMA/AAM surface-coated Pellethane 55D polyurethane, dextran surface-coated Pellethane 55D polyurethane and Blue Dextran surface-coated Pellethane 55D polyurethane samples from fresh human plasma (the results are expressed as the number of gold beads/cm²);

Fig. 17 is a graph showing ^{125}I -Albumin adsorption to Blue Dextran surface-coated Pellethane 55D polyurethane samples dried and rehydrated in PBS for 1 hour (the results are expressed as % of original albumin adsorption);

Fig. 18 is a graph showing the selective adsorption of albumin in the presence of fibrinogen to Pellethane 55D polyurethane and Blue Dextran surface-coated Pellethane 55D polyurethane samples (the results are expressed as the % of total protein adsorbed to the surface which is albumin);

Fig. 19 is a graph showing the % of adsorbed albumin removed from Pellethane 55D polyurethane and Blue Dextran surface-coated Pellethane 55D polyurethane samples during a 1% SDS wash;

Fig. 20 is a graph showing the competitive displacement of ^{125}I -albumin adsorbed on Blue Dextran surface-coated Pellethane 55D polyurethane and uncoated Pellethane 55D polyurethane samples washed in either PBS or PBS containing Blue Dextran (the results are expressed as % of albumin removed from samples during Blue Dextran wash);

Fig. 21 is a graph showing a comparison of ATIII activity of uncoated Pellethane 55D polyurethane, CBAS (heparin) coated Pellethane 55D polyurethane and Blue Dextran surface-coated Pellethane 55D polyurethane samples (the results are expressed as the amount of thrombin inactivated by the sample surfaces/cm²);

Fig. 22 is a graph showing percent decrease in bacterial adherence to Blue Dextran surface-derivatized Pellethane 55D compared to non-derivatized Pellethane 55D samples without albumin and with pre-albumin adsorption;

Fig. 23 is a schematic formula showing various monomers making up a polymer; and

Fig. 24 is a graph showing the amount of IU Thrombin inactivated on the surface of CBAS coated Pellethane 55D polyurethane and heparin coated Pellethane 55D polyurethane.

EXAMPLE I

Extruded Pellethane 55D polyurethane was used as a polyurethane material. It is available from the Dow Chemical Company of Midland, Michigan 48640, USA. Films of the 55D polyurethane were extracted in acetone for 72 hours and ethanol for another 72 hours prior to CelV ion grafting. The solvent extraction process removes any processing aids that might interfere with the grafting process. A 50% AMPS monomer solution in DI water was prepared and 20 ml of CelV ion solution per 100 ml of monomer solution was added. The CelV ion solution consisted of 2.74 g ceric ammonium nitrate and 3.15 g nitric acid in 50 ml DI water. The CelV-monomer solution was then degassed and released to nitrogen prior to grafting. Pellethane 55D polyurethane samples were placed into the degassed monomer solutions and stirred. Grafting was allowed to proceed for 2 hours. Grafted samples were then removed and thoroughly washed in DI water.

The presence of sulfonic acid groups on AMPS grafted material was measured using toluidine blue dye. Being positively charged, toluidine blue dye will ionically associate with negatively charged surfaces. Therefore, the binding of toluidine blue dye to the AMPS surface indicates the presence of negative charges due to the sulfonic acid groups in AMPS. AMPS grafted samples were therefore placed into a 1% toluidine blue dye/DI water solution for 1 minute and then rinsed in DI water. The bound dye was then released from the surface using a 1% sodium dodecylsulfate (SDS) in DI water solution. The amount of dye eluted was determined spectrophotometrically at 640 nm. The amount of dye released from Pellethane 55D samples and Pellethane 55D samples grafted with AMPS is shown in Fig. 1.

As the results indicate Pellethane 55D containing no AMPS adsorbed no toluidine blue dye. This is due to the fact that Pellethane 55D contains no negatively charged groups. However, the AMPS coating adsorbed a large amount of toluidine blue dye indicating the presence of sulfonic acid groups on the surface. Because the AMPS surface contained a large amount of sulfonation, its ability to bind ATIII was investigated next.

EXAMPLE 2

Since clotting may be retarded on AMPS surface coated materials by the activation of ATIII by the sulfonic acid groups present on the modified polymer substrate surface, the surface-mediated activation of ATIII by

AMPS coated samples was assessed. Samples were first rinsed in phosphate-buffered saline solution (PBS) for 15 minutes prior to ATIII exposure. Following rinsing, the samples were exposed for 15 minutes to an excess of purified ATIII (50 IU/ml). Non-adsorbed ATIII was removed by rapid rinsing in tris-buffered saline, pH 7.4 at 25°C (100 mM NaCl and 50 mM tris). The amount of surface bound and activated ATIII was then estimated by incubating the samples with an excess of thrombin. After a 10 minute incubation with constant mixing at 25°C, the residual thrombin was measured by reaction with a chromogenic substrate (H-D-phenylalanyl-L-pipecolyl-L-arginine-p-nitroanilide dichloride) in a spectrophotometer. The change in absorbance at 405 nm was then measured.

The results are given in Figure 2. As the results demonstrate, the AMPS surface derivatization appears to have some heparin-like activity. In fact, the AMPS coated samples appear to have more ATIII activity than CBAS® coated (Carmeda® Bioactive Surface) polyurethane samples. CBAS® is a heparin coating available from Carmeda AB, a Swedish company. This heparin-like effect is due to the sulfonic acid groups present in the AMPS coating. It is therefore expected that the AMPS coating will possess nonthrombogenic properties usually associated with heparin coated materials.

AMPS/GENTAMYCIN INFECTION-RESISTANT COATING

One major problem with implantable polymeric devices is their susceptibility to post-implant infection. Staph. epidermidis, which exists on human skin, and Staph. aureus, sometimes found in hospital environments, are the two most frequent pathogens. They both have the ability to enter the body through the surgical opening and attack an implant site. Though the chance of this happening can be minimized through good surgical techniques and clean operating room environment, it cannot be totally eliminated. Furthermore, the consequences of post-implant infection are almost always severe. Up to 5% of implants (depending on the device implanted) become infected; morbidity/mortality is most often the result.

Most prototype work to date on antimicrobial additions to AMPS surfaces has been done with gentamycin. It has been determined that it has the ability to ionically bond to the AMPS coating. Commercially, gentamycin is most frequently available as a sulfated salt. Since AMPS contains a sulfonic acid functionality, it was found that immersion of AMPS-coated polymer into a gentamycin sulfate solution resulted in the adsorption of gentamycin to the surface. Furthermore, in vitro, the adsorbed gentamycin eluted from the surface, thus giving the AMPS surface a bactericidal activity. As a result, the proper addition of antimicrobials to the AMPS coatings resulted in an infection-resistant as well as nonthrombogenic coating.

EXAMPLE 3

The procedure for loading the AMPS surfaces with gentamycin was as follows. An AMPS surface was created on a polymer substrate as described above. The AMPS-coated polymers were then immersed in an aqueous (normally 5% w/w) solution of gentamycin sulfate; this immersion may be for as little as 3 minutes. Upon completion of the immersion, the samples were removed, rinsed for 10-15 seconds in DI water, allowed to air dry, and stored.

Samples were analyzed for antibacterial activity using the "ring of inhibition" test. The ring of inhibition test is well known in the literature as an in vitro test of the antibacterial efficacy of a drug or drug-loaded system. It is set up and run as follows. A blood agar plate is cultured with the test bacterial organism and incubated overnight. One or two colonies from this plate are then swabbed and stirred into Tris-buffered saline (TBS) solution to a standard turbidity (1.5×10^9 /ml using Macfarland standards). This bacterial solution is then streaked on a Mueller-Hinton agar plate. The samples to be tested are then pressed onto or imbedded into the agar, along with one positive and one negative control. The positive control should be a drug-loaded sample with well-defined antibacterial properties; the negative control should be a sample containing no drug. One or two samples (depending on their size) can then be tested on the same plate. Once the agar is loaded with samples, it is turned upside down and incubated at 37°C overnight. The following day the plate may be removed from incubation and the ring of bacterial inhibition around each sample measured. The regions of bacterial growth and inhibition are obvious visually. The normal cloudy appearance of bacteria on agar is totally absent in the areas of inhibited growth. The ring of inhibition is simply measured with a ruler (if the "ring" is oblong, an approximate average radius of inhibition is taken). The ring of inhibition analysis is a good measure of the relative efficacy of various drug-loaded materials.

S. epidermidis was the test organism of all ring of inhibition testing done in connection with AMPS-coated polymers as described herein.

The graph of Fig. 3 demonstrates the bactericidal properties of AMPS coatings containing adsorbed gentamycin. Figure 3 shows the comparative efficacy vs. S. epidermidis of Pellethane 55D polyurethane, 55D

polyurethane with AMPS grafted coating, and AMPS coated Pellethane 55D polyurethane with adsorbed gentamycin.

As the results indicate in Figure 3, there were no bactericidal properties associated with the plain Pellethane 55D polyurethane or the AMPS grafted Pellethane 55D polyurethane, however, the AMPS grafted Pellethane 55D polyurethane containing adsorbed gentamycin demonstrated a significant bactericidal activity. The ability of the gentamycin to elute from the AMPS grafted surfaces under different ionic conditions was investigated next.

Figure 4 shows the results of an elution test done to determine how ionic strength affects the rate of elution of gentamycin from the AMPS coating. Note that the greater the ionic strength of the storage solution, the faster the ring of inhibition decreases, and therefore, the faster the rate of gentamycin elution from the surface. This not only indicates that ionic bonding of drug to coating has occurred, but that at a physiological ionic strength (0.9% concentration), the rate of desorption is conducive to a long-term, slow release of drug. These kinetics are remarkably similar to those of diffusion of drug from a bulk-loaded matrix, indicating that therapeutic amounts of drug will release over a relatively long period of time.

EXAMPLE 4

The following Example was prepared to determine if the adsorption of gentamycin would decrease the ATIII activity of an AMPS coated surface. It consists of loading AMPS grafted surfaces with gentamycin via immersion in an aqueous (normally 5% w/w) solution of gentamycin sulfate for 5 minutes. Upon completion of the immersion in gentamycin, the samples were removed, rinsed for 10-15 seconds in DI water and either dried or placed into a 10% NaCl solution for 24 hours. Samples were then analyzed for ATIII activity.

Samples were first rinsed in phosphate-buffered saline (PBS) for 15 minutes prior to ATIII exposure. Following rinsing, the samples were exposed for 15 minutes to an excess of purified ATIII (50 IU/ml). Non adsorbed ATIII was removed by rapid rinsing in tris-buffered saline, pH 7.4 at 25°C (100 mM NaCl and 50 mM tris). The amount of surface bound and activated ATIII was then estimated by incubating the samples with an excess of thrombin. After a 10 minute incubation with constant mixing at 25°C, the residual thrombin was measured by reaction with a chromogenic substrate (H-D-phenylalanyl-L-pipecolyl-L-arginine-p-nitroanilide dichloride) in a spectrophotometer. The change in absorbance at 405 nm was then measured. The results are given in Figure 5. As the results demonstrate, the AMPS surface which contains adsorbed gentamycin retains a large percentage (74%) of its original ATIII activity. The ATIII activity which the AMPS surface loses due to the adsorbed gentamycin returns upon the elution of the gentamycin. It is concluded, therefore, that the AMPS coating possesses most of its antithrombogenic properties while loaded with gentamycin and regains all of its antithrombogenic properties upon the elution of gentamycin. Therefore, such a coating is nonthrombogenic and bacterial resistant.

The idea behind the AMPS coating is to create a heparin-like surface to prevent thrombogenicity with the added benefit of providing slip properties to implantable devices. The data discussed above demonstrate that the coating does indeed create a heparin-like surface by virtue of this selective adsorption of ATIII. In addition to these antithrombogenic properties, it was discovered that the anionic AMPS coating has the ability to ionically bind and slowly release cationic antimicrobials thus imparting long-term bioactive properties to the surface as well. It was found that this addition of bioactive agents did not adversely affect the ability of the AMPS surface to selectively adsorb ATIII.

These results indicate that the AMPS coating is an effective nonthrombogenic as well as infection-resistant surface modifier significantly increasing the biocompatibility of implantable polymeric medical devices.

Other examples of cationic antimicrobials [attracted to (-) charged surfaces] are shown in Table 3 below.

TABLE 3

	Gentamycin
5	Kanamycin
	Neomycin
	Silver ion
10	Chlorhexidine
	Vancomycin
	Streptomycin
15	Erythromycin

Specific examples of anionic antimicrobials [attracted to (+) charged surfaces] are shown in Table 4 below.

TABLE 4

20	Ampicillin
	Oxacillin
	Cefazolin
25	Bacitracin
	Cephalosporin
	Cephalothin
30	Cefuroxime
	Cefoxitin
	Norfloxacin
35	Perfloxacin
	Sulfadiazine

40 BASIC FIBROBLAST GROWTH FACTOR BINDING TO IONICALLY CHARGED SURFACES (GRAFT POLYMER)

Basic fibroblast growth factor (bFGF) has been isolated from many tissue sources and is a single chain protein with an approximate molecular weight of 18,000. It is a cationic protein with an isoelectric point of 9. The binding of bFGF with ionically charged surfaces is demonstrated below.

Polyurethane samples used herein possessed either negatively charged, positively charged or neutrally charged hydrophilic graft polymer surfaces. The modified polyurethane surfaces were prepared via a ceric ion grafting. Noncoated polyurethane samples (PU) were also used.

50 EXAMPLE 5

Neutrally Charged Samples (AAm)

Neutrally charged polyurethane samples (1 cm²) were prepared by placing the samples into the following degassed grafting solution for 45 minutes at ambient temperature.

50 g acrylamide (AAm)
1.1g ceric ammonium nitrate

1.3 g nitric acid
 70 ml deionized (DI) water
 Following grafting, the samples were rinsed thoroughly in DI water.

5 EXAMPLE 6

Positively Charged Samples (APMA) (AAM)

Positively charged polyurethane samples (1 cm²) were prepared by placing the samples into the following degassed grafting solution for 45 minutes at ambient temperature.

40 g acrylamide (AAM)
 10 g N-(3-aminopropyl)methacrylamide hydrochloride (APMA)
 1.1 g ceric ammonium nitrate
 1.3 g nitric acid
 70 ml DI water

Following grafting, the samples were rinsed thoroughly in DI water.

EXAMPLE 7

20 Negatively Charged Samples (AMPS)

Negatively charged polyurethane samples (1 cm²) were prepared via placing the samples into the following degassed grafting solution for 2 hours at ambient temperature.

50 g 2-acrylamido-2-methylpropane sulfonic acid (AMPS)
 1.1 g ceric ammonium nitrate
 1.3 g nitric acid
 70 ml DI water

Following grafting, the samples were rinsed thoroughly in DI water.

30 RESULTS - EXAMPLE 5 - 7

The grafted samples (1 cm²) were then placed into 24 well polystyrene tissue culture plates and secured in place with silicone rubber rings. The samples were stored hydrated in water at ambient temperature until assayed.

35 Diluted bFGF in phosphate buffered saline (PBS) with 1 mg/mL bovine serum albumin (BSA). Incubated samples overnight at 25°C. Three concentrations of bFGF were studied: 0.5 ng/mL, 5 ng/mL and 50 ng/mL. 0.1mg ¹²⁵I- labeled bFGF were added to each well. 0.5 mL total volume per well. The samples were washed with PBS. The samples were counted in a Beckman gamma counter.

TABLE 5

RESULTS:	
Treatment :	CPM
PU (control)	8971
#5 AAM	6721
#6 APMA	4113
#7 AMPS	29931

As can be seen from the data in Table 5 and Figure 6, the amount of fibroblast growth factor which binds to the various surfaces is demonstrated. As the results indicate the negatively charged samples (AMPS) adsorbed considerably more bFGF than did the other samples.

55 On the other hand, if one wanted to achieve the opposite result i.e., repulsion as opposed to attraction; one would select a graft polymer/bioagent having similar charges (+,+) or (-,-). This might be done in those instances where one wishes to minimize any interaction therebetween. For example, one may wish to minimize

peptide interaction with respect to an implanted surface. To accomplish this one may graft hydrophylic polymers on the implanted surface that contains similar charges to the peptide of interest thus enhancing the lack of interaction through charge repulsion. As an example, consider Figure 6, in which one could select an APMA/AAm coating to repulse bFGF. Consider Figure 7 in which one could select an APMA/AAm coating to repulse toluidine blue. Lastly consider Figure 8 in which one could select an AMPS coating to repulse Ponceau S.

IONIC BINDING OF DYES TO CHARGED SURFACES (GRAFT POLYMER)

The ability of a cationic and an anionic dye to bind to charged surfaces was also investigated. This is of interest because of the concept of utilizing various dyes as biological ligands. The cationic dye used in this study was Toluidine Blue dye and the anionic dye used was Ponceau S dye.

Polyurethane substrate samples used herein possessed either negatively charged, positively charged or neutrally charged hydrophilic surfaces. The polyurethane surfaces were prepared via a ceric ion grafting. Uncoated polyurethane samples (PU) were also used.

EXAMPLE 8

Neutrally Charged Samples (AAm)

Neutrally charged polyurethane samples (1 cm²) were prepared via placing the samples into the following degassed grafting solution for 45 minutes at ambient temperature.

- 50 g acrylamide (AAm)
- 1.1 g ceric ammonium nitrate
- 1.3 g nitric acid
- 70 ml deionized DI water

Following grafting, the samples were rinsed thoroughly in DI water.

EXAMPLE 9

Positively Charged Samples (APMA/AAm)

Positively charged polyurethane samples (1 cm²) were prepared via placing the samples into the following degassed grafting solution for 45 minutes at ambient temperature.

- 40 g acrylamide (AAm)
- 10 g N-(3-aminopropyl)methacrylamide hydrochloride (APMA)
- 1.1 g ceric ammonium nitrate
- 1.3 g nitric acid
- 70 ml DI water

Following grafting, the samples were rinsed thoroughly in DI water.

EXAMPLE 10

Negatively Charged Samples (AMPS)

Negatively charged polyurethane samples (1 cm²) were prepared via placing the samples into the following degassed grafting solution for 2 hours at ambient temperature.

- 50 g 2-acrylamido-2-methylpropane sulfonic acid (AMPS)
- 1.1 g ceric ammonium nitrate
- 1.3 g nitric acid
- 70 ml DI water

Following grafting, the samples were rinsed thoroughly in DI water.

RESULTS - EXAMPLES 8 - 10

Grafted samples of Examples 8 - 10 (1 cm²) were placed into either a 1% Toluidine Blue dye in DI water solution (12a-14a) or a 1% Ponceau S dye in DI water solution (12b-14b) for 1 minute. Following dyeing the samples were rinsed thoroughly in 500 ml DI water. Dyed samples were agitated in a 1% sodium dodecyl sulfate

(SDS) in DI water solution for 24 hours.

The absorbance of each SDS sample solution containing eluted dye was then measured on a Beckman DU 64 spectrophotometer at a wavelength of 640 nm for Toluidine Blue dye and 520 nm for Ponceau S dye.

The amount of dye adsorbed to each sample is then calculated by comparing the sample absorbance to an absorbance standard curve generated using known amounts of dye. The results for Toluidine blue dye adsorbance are shown in Table 6, as well as Figure 7.

TABLE 6

Toluidine Blue Dye Adsorbance	
Treatment:	mg Toluidine Blue Adsorbed/cm ²
PU (Control)	2.17
#8a AAm	1.52
#9a APMA/AAm	1.58
#10a AMPS	96.07

Similar results are shown in Table 7 and Figure 8 for Ponceau S dye adsorbance.

TABLE 7

Ponceau S dye Adsorbance	
Treatment:	mg Ponceau S Adsorbed/cm ²
PU (Control)	1.90
#8b AAm	2.24
#9b APMA/AAm	29.62
#10b AMPS	0.24

As the results indicate, the negatively charged samples (AMPS) adsorbed considerably more of the cationic dye (Toluidine Blue) than did the other samples. Whereas, the positively charged samples (APMA/AAm) adsorbed considerably more of the anionic dye (Ponceau S) than did the other samples.

COVALENT BINDING OF BIOACTIVE AGENTS

The concept of covalently binding bioactive agents such as dyes onto implantable materials, such as polyurethane, having grafted slip coatings is another aspect of this invention. Materials which have surfaces that preferentially bind albumin will be less thrombogenic, less pro-inflammatory and less liable to harbor adherent pathogenic bacteria.

The present invention describes herein techniques for covalently attaching Blue Dextran, dextran or Cibacron Blue dye to a grafted polymer surface. The binding of these agents is based on the generation of free radicals on a base material surface and the copolymerization of vinyl monomers directly to that surface followed by covalent attachment of bioactive agents i.e., Blue Dextran, dextran or Cibacron Blue dye. A number of techniques have been reported in the area of graft copolymerization on materials. Graft copolymerization provides a method of controlling the composition of the grafted polymer by varying monomers and varying their concentrations, it is possible to create grafted surfaces that possess the following properties.

1. Hydrophilicity.
2. No non-specific interactions with proteins in general.
3. A sufficient number of active groups that are amenable to the chemical functionalization and modification required in covalently attaching bioactive agents e.g., Blue Dextran, dextran or Cibacron Blue dye.
4. Mechanical, chemical and enzymatic stability.

Grafting can be engineered stoichiometrically to produce surfaces containing the desired amount of hydrophilicity and the desired amount of functional groups used for coupling the bioactive agents. Hydrophilic

spacer monomers (neutral, anionic and cationic) can be selected to produce appropriately charged hydrophilic spacers. Coupling monomers can be selected based on pendant functional groups, i.e., -OH, -NH₂, -CHO, -NCO, needed or wanted for immobilizing a bioactive agent e.g., Blue Dextran, Dextran or Cibacron Blue dye.

Graft copolymerizations onto poly(ether urethane) with CeIV ion has been the mechanism utilized. The monomers utilized are acrylamide (AAM) and N-(3-aminopropyl) methacrylamide hydrochloride (APMA). These monomers were chosen based partly on their reactivity and water solubility characteristics. The AAM monomer is used as the hydrophilic spacer monomer. The APMA monomer is used as the coupling monomer. AAM has been used extensively as an affinity chromatographic matrix. AAM is a neutral hydrophilic monomer; therefore, polymers of AAM have shown little protein association. APMA is an amino containing monomer, thus allowing the attachment of Blue Dextran, dextran or Cibacron Blue dye.

Cibacron Blue dye can be reacted directly to the amino functional group on the APMA monomer through its reactive chlorine on its triazine ring. Dextran and Blue Dextran can be covalently attached to the amino group on the APMA through a stabilized Schiff base reaction. The dextran or Blue Dextran is first oxidized using sodium periodate. The oxidation forms reactive aldehyde groups which react with the primary amines on APMA forming imines. The imines are stabilized using sodium cyanoborohydride.

Cibacron Blue dye can be reacted to the covalently attached dextran forming Blue Dextran on the surface of the material. This gives the ability of attaching as much dye as desired on to the surface. Also, the dextran that is attached can be varied in molecular weight as desired.

BLUE DEXTRAN SURFACE COATING TECHNIQUE

The Blue Dextran surface-derivatization technique begins with the graft copolymerization of AAM and APMA monomers onto a clean polyurethane surface with CeIV ion. The CeIV ion creates a free radical on the polyurethane surface which initiates the graft copolymerization of the acrylamides (Figures 9a and b). The APMA monomer contains a primary amino group that is then used for coupling Dextran which is later dyed with Cibacron Blue dye. The attachment of Dextran consists of first oxidizing the carbohydrate with sodium periodate thus forming aldehyde groups (Figure 10). These aldehyde groups are then covalently attached to the primary amino group on the grafted APMA monomer through reductive amination (Schiff base reaction) (Figure 11). Sodium cyanoborohydride (NaCNBH₃) is used to stabilize the imine linkage (Figure 12). Finally, Cibacron Blue dye is then covalently coupled to the surface-bound dextran (Figure 13).

The amount of surface amination (the graft copolymerization of APMA and AAM) that takes place on the polyurethane surface can be effected via monomer concentrations, catalyst concentration, and grafting time. Therefore, a study was performed to determine the optimum grafting conditions for achieving a highly aminated polyurethane surface. The amount of surface amination achieved is measured via staining the surface with Ponceau S dye, a negatively charged dye molecule. The dye ionically associates with primary amines on the aminated surface. After staining, the dye is released from the surface using SDS and quantified spectrophotometrically at 520 nm.

EXAMPLE 11

APMA/AAM Grafting Procedure

Extruded Pellethane 55D films were extracted in acetone and ethanol prior to CeIV ion grafting. The solvent extraction process removes any processing aides that could interfere with the grafting process. Monomer solutions in DI water were prepared and various amounts of CeIV ion solution were added. The CeIV ion solution consisted of 2.74 g. ceric ammonium nitrate and 3.15 g. nitric acid in 50 ml DI water. The CeIV - monomer solutions were degassed and released to nitrogen prior to grafting. Pellethane samples were placed into the degassed monomer solutions and stirred. Grafting was allowed to proceed for various amounts of time. Samples were then removed and thoroughly washed in DI water.

The amount of surface amination that took place upon graft copolymerization of the samples was measured using Ponceau S dye. Grafted samples were placed into a 1% Ponceau S dye/DI water solution for 1 minute and then rinsed in DI water. The bound dye was then released from the surface using a 1% SDS solution. The amount of dye eluted was determined spectrophotometrically at 520 nm.

Table 8 describes the various grafting conditions employed and the amount of surface amination achieved as determined by the Ponceau S staining technique. Based on these results samples were grafted using a total monomer concentration of 50%, a reaction time of 30 minutes, a catalyst concentration of 20% and an APMA/AAM monomer ratio of 0.10. (10/90%).

EXAMPLE 12**Dextran Attachment to Aminated Polyurethane Surfaces**

- 5 Dextran was first oxidized by placing 1.0 g in 18 g DI water containing 1.0 mg NaIO_4 . The mixture was incubated in the dark for 2 hours. The oxidized mixture was then dialyzed against DI water for 24 hours to remove any excess periodate. APMA/AAm grafted Pellethane 55D samples were then placed into the oxidized dextran solution for one hour at ambient temperature followed by the addition of NaCNBH_3 (3 mg/ml). The resultant mixture was allowed to react for 2 hours at ambient temperature. The samples were then removed and
10 rinsed thoroughly in DI water.

EXAMPLE 13

- Samples containing covalently coupled dextran were then dyed with Cibacron Blue dye.
15 Samples were placed into a dye solution consisting of 1.0 g Cibacron Blue dye, 4.0 g NaCl, 12.0 g NaHCO_3 in 200 ml DI water overnight. Blue Dextran samples were then thoroughly rinsed in DI water.

SEM Analysis of Blue Dextran Surface-Coated Material

- 20 (Example 13)
Scanning electron microscopy was performed on surfaces of Blue Dextran surface-coated Pellethane 55D. The surfaces were investigated at a magnification of 6000X. SEM photographs of the surface indicate the Blue Dextran/APMA/AAm coating appears to be approximately 2 microns thick and uniformly distributed
25 on the surface.

30

35

40

45

50

55

Table 8. Surface Amination of Pellethane 55D Films.

	<u>Monomer</u>	<u>Rxn Time</u>	<u>Catalyst %</u>	<u>APMA/AAM</u>	<u>Ponceau S</u> <u>Abs.</u>
5					
	50	30	20	0.10	0.911
10					
	50	30	10	0.10	0.770
15					
	50	30	40	0.10	0.655
	50	30	5	0.10	0.547
20					
	50	30	60	0.10	0.516
	50	30	3	0.10	0.084
25					
	50	30	1	0.10	0.004
	50	15	20	0.10	0.590
30					
	50	15	60	0.10	0.395
35					
	50	15	10	0.10	0.368
	50	15	5	0.10	0.331
40					
	50	15	40	0.10	0.300
	50	15	3	0.10	0.075
45					
	50	15	1	0.10	0.006
50					
	50	5	20	0.10	0.150
	50	5	60	0.10	0.144
55					

	<u>Monomer</u>	<u>Rxn Time</u>	<u>Catalyst %</u>	<u>APMA/AAM</u>	<u>Ponceau S</u> <u>Abs.</u>
5	50	5	40	0.10	0.137
	50	5	10	0.10	0.109
10	50	5	5	0.10	0.041
	50	5	3	0.10	0.010
15	50	5	1	0.10	0.006
20	40	15	3	0.05	0.367
	40	15	5	0.05	0.363
25	40	15	1	0.05	0.017
	30	15	3	0.05	0.310
30	30	15	5	0.05	0.232
35	30	15	1	0.05	0.030
	20	15	3	0.05	0.328
40	20	15	5	0.05	0.217
	20	15	1	0.01	0.091
45	20	5	3	0.05	0.060
50	20	5	1	0.05	0.041

ESCA Analysis of Blue Dextran Surface-Coated Material

ESCA was done on uncoated Pellethane 55D samples (control), APMA/AAM surface-coated Pellethane 55D samples, Dextran surface-coated Pellethane 55D samples and Blue Dextran surface-coated Pellethane 55D samples (Table 9). As the results indicate, Blue Dextran is present on the Blue Dextran surface-coated material's surface. This is demonstrated by the decrease in carbon, an increase in oxygen and an increase in sulfur.

TABLE 9

ESCA analysis of Blue Dextran surface-coated 55D (Blue Dextran), Dextran surface-coated 55D (Dextran), APMA/AAm surface-coated 55D (APMA/AAm) and uncoated Pellethane 55D (55D). Theoretical percentages are also given for Blue Dextran (1 dye/10 sugars).

SAMPLE	%CARBON	%OXYGEN	%NITROGEN	%SULFUR
55D (control)	78.50	15.90	3.90	0.00
#11 APMA/AAm	67.84	19.70	12.46	0.00
#12 Dextran	66.16	20.21	13.63	0.00
#13 Blue Dextran	62.36	20.76	12.91	1.43
BD _{theoretical}	52.80	40.99	4.35	1.86

Tensile Properties of Blue Dextran Surface-Coated Material

Tensile properties of Blue Dextran surface-coated Pellethane 55D films, dextran surface-derivatized Pellethane 55D films, APMA/AAm surface-derivatized Pellethane 55D films and Pellethane 55D films were measured using an Instron mechanical testing apparatus. As shown in Table 10, the graft copolymerization step and further derivatization steps of the Blue Dextran surface derivatization technique do not substantially alter the physical properties of the polyurethane.

TABLE 10

Tensile Properties of Blue Dextran surface-coated Pellethane 55D, Dextran surface-coated Pellethane 55D, APMA/AAm surface-coated Pellethane 55D and uncoated Pellethane 55D films.

SAMPLE	UTS* MPa (psi)	% ELONGATION
55D	52.2 ± 9.9 (7570 ± 1440)	361 ± 35
#11 APMA/AAm	54.8 ± 5.1 (7950 ± 736)	444 ± 37
#12 Dextran	48.5 ± 4.4 (7031 ± 634)	438 ± 38
#13 Blue Dextran	46.9 ± 6.0 (6806 ± 875)	390 ± 40

* Ultimate Tensile Strength

EXAMPLE 14**Th Adsorption of Albumin on Surface-Coated Material**

The extent of binding of radiolabeled albumin to Blue Dextran surface-coated samples, Blue Dextran bulk-loaded samples (55D/BD) and Pellethane 55D samples (control) were studied. The samples were rinsed in phosphate buffered saline (PBS) for 15 minutes each prior to incubation in ^{125}I -albumin (0.08 mg/ml) for 15 minutes. The samples were then removed and washed again in PBS and counted in a scintillation counter. The results (Figure 14) indicate that the Blue Dextran surface-coated samples adsorbed more albumin than the control 55D samples and more than the bulk incorporated 55D/BD samples.

EXAMPLE 15**The Adsorption of Albumin on the Surface-Coated Blue Dextran**

The extent of binding of radiolabeled albumin to Blue Dextran surface-coated samples, Dextran surface-coated samples, APMA/AAm surface-coated samples, and uncoated 55D samples was studied. This study was performed to determine if the binding of albumin was due to the presence of Cibacron Blue dye in the Blue Dextran coating or instead to the underlying dextran or APMA/AAm coatings. Samples were rinsed in PBS for 15 minutes each prior to incubation in ^{125}I -albumin (0.08 mg/ml) for 15 minutes. The samples were then removed and washed again in PBS and counted in a scintillation counter. The results (Figure 15) indicate that the Blue Dextran surface-coated samples adsorbed considerably more albumin than the other samples. This increase in albumin adsorption is therefore due to the presence of the Cibacron Blue dye in the Blue Dextran coating.

EXAMPLE 16**Immunogold Assay of Albumin Adsorbed to Surface-Coated Material**

Another study was performed to determine if the binding of albumin was again due to the presence of Cibacron Blue dye in the Blue Dextran coating or instead to the underlying dextran or APMA/AAm coatings. In this study, samples were rinsed in PBS for 15 minutes each prior to incubation in fresh human plasma for 45 minutes at 37°C. Samples were then rinsed in PBS for 5 minutes. Following rinsing, the samples were placed into a 1.0% glutaraldehyde solution for 7 minutes and then rinsed in PBS for 10 minutes. Samples were then placed into 1 ml of 50 mM glycine solution for 7 minutes. The samples were then rinsed again in PBS for 10 minutes. Following rinsing, the samples were incubated in 1 ml of 15% milk solution containing 10 µg of Protein A solution for 1 hour at 37°C. Samples were then rinsed in PBS for 10 minutes. Following rinsing, the samples were placed in 0.5 ml of rabbit antihuman albumin and incubated for 1 hour at 37°C. Samples were then rinsed in PBS for 10 minutes. Following rinsing, the samples were incubated overnight in 1 ml of 2.5% glutaraldehyde solution at 4°C. After incubation in the glutaraldehyde solution the samples were rinsed in PBS for 10 minutes. Samples were then placed in 1 ml of 50 mM glycine solution for 25 minutes followed by a 1 hour incubation in 0.5 ml of gold labeled protein A solution at 37°C. Samples were then rinsed in PBS for 10 minutes and DI water for 10 minutes. Silver enhancement solution (0.5 ml) was then added to the samples for 15 minutes. Samples were removed and their surfaces were photographed with a scanning electron microscope. The number of beads on the SEM photos of the surfaces of APMA/AAm surface-derivatized 55D, dextran surface-derivatized 55D and the Blue Dextran surface-derivatized 55D were counted and tabulated in Figure 16.

As the results indicate there is not a significant difference between dextran and APMA/AAm surfaces; however, there is significantly more albumin on Blue Dextran surfaces.

EXAMPLE 17**Drying and Rehydration Effects on Albumin Binding**

There were some concerns that, if the Blue Dextran Surface dried out, it might lose some of its albumin binding activity since the surface is extremely hydrophilic. Therefore, dehydration/rehydration studies were run. Blue Dextran surface-coated Pellethane 55D samples were dried out in an oven at 50°C and then tested for albumin adsorption. Some of the dried samples were rehydrated in PBS for one hour and then tested for albumin adsorption. Albumin adsorption tests consisted of incubation of samples in ^{125}I -albumin (0.08 mb/m.) for 15 minutes followed by rinsing in PBS and counting in a scintillation counter. As the results in Figure 17 indicate

drying out of the surface decreases its albumin activity, however, rehydration of dried samples appears to revive them.

EXAMPLE 18

The Selective Binding of Albumin by Surface-Coated Material

The selective binding of albumin to grafted Blue Dextran surfaces in the presence of fibrinogen was determined next using 50/50 mixtures of albumin/ ^{125}I -fibrinogen and ^{125}I -albumin/fibrinogen. Fibrinogen was present to determine the nonspecific binding of proteins to the surface and because surfaces which avidly adsorb fibrinogen may be highly thrombogenic. Therefore, samples were exposed to an albumin/ ^{125}I -fibrinogen or ^{125}I -albumin/fibrinogen mixture for 15 minutes and then rinsed with PBS. After the PBS rinsing, the samples were counted in a scintillation counter. As shown in Figure 18, the Blue Dextran surface-coated samples had a greater ability to selectively adsorb albumin despite the presence of fibrinogen. The reversibility of albumin adsorbed to Blue Dextran surface-coated material was tested next.

EXAMPLE 19

The Reversible Binding of Albumin to Surface-Coated Material

The reversible binding of albumin was determined by studying its elutability with a 1% SDS wash. Protein that remains adherent to a surface following a SDS wash is considered denatured and irreversibly bound (see Rapoza et al J. Biomedical Materials Research 24: 1263-1287 (1990)).

Therefore, samples were incubated for five hours in ^{125}I -albumin then removed and washed in a 1% SDS solution for one hour. As Figure 19 shows, approximately 95% of the albumin initially adsorbed to Blue Dextran grafted surfaces was removed during the SDS wash. These results indicate adsorbed albumin does not denature and is reversibly bound on the Blue Dextran surface-coated samples. The binding site of albumin on Blue Dextran surface-coated samples was tested next.

EXAMPLE 20

The Binding Site of Albumin on Surface-Coated Material

The binding site of albumin to Blue Dextran surface-coated Pellethane 55D was investigated by using sol-phase Blue Dextran to competitively block albumin binding to the Blue Dextran grafted to the surface of the polyurethane. Blue Dextran surface-coated and uncoated Pellethane 55D samples were rinsed in PBS for 15 minutes each prior to incubation in ^{125}I -albumin (0.08 mg/ml) for 15 minutes. The samples were then washed in either PBS or PBS containing soluble Blue Dextran and counted in a scintillation counter. Results are given in Figure 20. As can be seen in Figure 20, the inclusion of Blue Dextran in the wash buffer selectively removed approximately 95% of the albumin adherent to surface-coated material. Therefore, albumin bound to surface-coated material is associated with the Blue Dextran on the surface.

EXAMPLE 21

Blood Loop Assay of Blue Dextran Surface-Coated Material

Pellethane 55D (2 mm in diameter and 30 cm in length) was surface-coated with Blue Dextran. A venipuncture was performed on a human subject without anticoagulant into a plastic syringe. One-milliliter aliquots of whole blood were then delivered immediately into a 12 x 75 mm glass test tube, an uncoated Pellethane 55D tubing sample. One end of each polyurethane tube was looped around and connected to the other end via a silicone connector. A stopwatch was started as soon as the blood entered the samples. The polyurethane tubes were then rotated at 9 rev/min until the blood clotted. The glass test tube was gently tilted every 30 seconds until a clot was seen; the stopwatch was stopped at these points and the time was recorded. The rate at which 1 ml of fresh whole blood clotted in the polyurethane tubes was compared to the rate of clotting in the glass tube. Results are shown in Table 11.

TABLE 11

Whole blood clotting time of a glass test tube, uncoated Pellethane 55D tubing and Blue Dextran surface-coated Pellethane 55D tubing.	
SAMPLE	CLOTTING TIME
Glass	5 mins 32 secs
Uncoated Pellethane 55D	9 mins 43 secs
Blue Dextran surface-coated Pellethane 55D	>16 hours

The results in Table 11 indicate that both Blue Dextran surface-coated and uncoated tubing surfaces had an inhibitory effect on whole blood coagulation. However, the tubing that contained Blue Dextran had a large enough effect as to prevent complete clotting during the 16 hours it was allowed to rotate. Therefore, Blue Dextran surface-coating evidently decreases the thrombogenicity of Pellethane 55D.

EXAMPLE 22

Following the clotting study, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on the polyurethane tubes to determine if there was an albumin coating on the surface of Blue Dextran surface-coated material. Both polyurethane surfaces were vigorously rinsed in PBS. The tubes were then cut into two pieces. One piece of each tube was placed in SDS-PAGE buffer solution and incubated overnight to remove the adherent protein. The buffer solution consisted of 62.5 mM Tris-HCl, 5% β -Mercaptoethanol, 10% glycerol and 2.3% SDS in DI water. SDS-PAGE was then performed on 100 μ l of buffer solution containing the eluted proteins. Following electrophoresis, the gel was stained with Coomassie Brilliant Blue, and the identity of the eluted proteins was determined by reference to molecular weight standards included on the gel. The results of this experiment indicate that the major protein adsorbed to Blue Dextran surface-coated material is albumin. By contrast, uncoated material had much less adsorbed albumin and proportionately more non-albumin proteins.

EXAMPLE 23

The other piece of each of the polyurethane tubings was placed in 2.5% glutaraldehyde solution overnight. Scanning electron microscopy was then used to examine the surface of each of the tubings. The SEM photographs demonstrated a lot of thrombus on the surface of the uncoated material. In contrast, there was no thrombus formation on the Blue Dextran coated surface. In fact, there was no apparent cellular adhesion of any kind. Therefore, the Blue Dextran surface coating technique decreased the thrombogenicity of the polyurethane surface. Next, the Blue Dextran surface coating was tested to see if it had any heparin-like activity.

EXAMPLE 24

ATIII Activity of Blue Dextran Surface-Coated Material

Clotting may be retarded on Blue Dextran surface-coated materials by the activation of ATIII by the three sulfonic acid groups per molecule of dye. This interaction may cause a heparin-like activation of ATIII.

Therefore, the surface-mediated activation of ATIII by coated samples was assessed. Samples were first rinsed in PBS for 15 minutes prior to ATIII exposure. Following rinsing, the samples were exposed for 15 minutes to an excess of purified ATIII (50 IU/ml). Non-adsorbed ATIII is removed by rapid rinsing in tris-buffered saline, pH 7.4 at 25°C (100 mM NaCl and 50 mM tris). The amount of surface bound and activated ATIII is then estimated by incubating the samples with an excess of thrombin. After a 10 minute incubation with constant mixing at 25°C, the residual thrombin was measured by reaction with a chromogenic substrate (H-D-phenylalanyl-L-pipecoly-L-arginine-p-nitroanilide dichloride) in a spectrophotometer. The change in absorbance at 405 nm was then measured. The results are given in Figure 21. As the results demonstrate Blue Dextran surface coating appears to have some heparin-like effect. This heparin-like effect is presumably due to the Cibacron Blue dye in the Blue Dextran coating.

EXAMPLE 25

Bacterial Adherence to Blue Dextran Surface-Coated Material

5 The adherence of pathogenic bacteria to Blue Dextran surface coated and uncoated Pellethane 55D was investigated to determine the effectiveness of the Blue Dextran surface coating technique in preventing bacterial adherence. Staphylococcus epidermidis were chosen for the bacterial adhesion study since they are the most frequent cause of medical device associated infections.

10 Staph. epidermidis were grown overnight in Brain Heart Infusion. The bacteria were concentrated centrifugally and washed three times in isotonic saline solution. Bacteria were resuspended to 7×10^8 cfu/ml and samples of Blue Dextran surface-coated and uncoated Pellethane 55D were either immersed in the suspension with gentle mixing for two hours at 25°C or pre-incubated in an albumin solution for 15 minutes followed by the two hour incubation in the bacterial solution. After incubation the samples were rinsed in sterile isotonic saline solution and then placed in 5 ml of sterile saline. The samples were then sonicated at 20 watts for one minute. After sonication 50 µl of solution, now containing displaced bacteria, were plated, incubated and counted. The results are given in Figure 22.

The samples were stained and examined under a light microscope after sonication. There appeared to be no adherent bacteria left on the samples upon examination under the light microscope, indicating the sonication had knocked off the adherent bacteria.

20 As the results in Figure 22 indicate Blue Dextran surface coated material adhered approximately 50% fewer bacteria than did the uncoated material with no albumin present. This decrease is presumably due to the hydrophilic surface of the coated material. In the presence of albumin however, the Blue Dextran surface-coated material was able to decrease the bacterial adherence by almost 100%.

25 Summary of Blue Dextran Surface-Coating Technique

The Blue Dextran surface coating technique consists of the graft copolymerization of APMA/AAM to the polyurethane surface using CeIV ion. Dextran is then covalently attached to this hydrophilic coating through a Schiff base reaction. Cibacron Blue dye is then covalently attached to the bound dextran forming the Blue Dextran surface-coated polyurethane.

30 The binding of albumin to surface-coated material was shown to be approximately 95% specific and occurs even in the presence of fibrinogen. Second, it appears that the albumin which adsorbs to uncoated material has denatured and cannot be competitively displaced. In contrast, 95% of the albumin bound to surface-coated material is readily displaced by a 1% SDS solution. Third, the binding of albumin to 95% of the surface-coated material's surface is evidently mediated primarily through site-specific binding of the protein to the Blue Dextran. This is supported by the observation that added (free) blue dextran will competitively release approximately 95% of the albumin pre-adsorbed to surface coated material.

40 The binding of albumin to Blue Dextran surface-coated material was shown to inhibit the surface activation of clotting, as measured by the whole blood clotting time, decrease the adherence of blood platelets and prevent the formation of fibrin on the surface. The Blue Dextran surface coating technique also was shown to adhere significantly fewer bacteria.

45 Thrombosis and infection are probably the two greatest hindrances to the utilization of artificial implantable medical devices. The materials of these devices tend to cause the formation of thrombi and serve as a focus for infection of the body by a number of bacterial species. Device-associated infections are promoted by the tendency of these organisms to adhere and colonize the surface of the device. Thrombus formation can be promoted by the adsorption of large amounts of cell adhesion proteins, i.e, fibrinogen, to the device surface. This adsorbed adhesive protein then interacts with the platelet membrane GPIIb-IIIa receptors and possibly GPIb receptors leading to platelet deposition and activation. The deposition and activation of platelets causes platelet aggregation and finally thrombus formation. To prevent thrombus formation and infection from occurring it was determined that an implantable material should possess an "active" surface which selectively and reversibly binds without denaturing albumin with high affinity. Therefore, a modification technique for increasing the albumin-binding ability of an implantable polyurethane was created.

55 The technique creates a biomaterial surface comprised of a high affinity albumin-binding dye to which albumin selectively and reversibly binds forming a renewable endogenous albumin coating on the surface when the material is in contact with a physiological fluid containing albumin. The formation of this albumin coating was demonstrated to diminish the tendency of the material to promote thrombosis on the material surface by preventing the cell adhesion proteins and the pro-coagulation proteins from adsorbing to the material surface. The coating also was demonstrated to diminish the tendency of the material to promote bacterial infection by

decreasing the adherence and colonization of pathogenic bacteria to the material surface.

The modification technique provides a method of which approximately 95% of the material's surface selectively and reversibly binds albumin.

5 HEPARIN COATING

Grafting can be engineered stoichiometrically to produce surfaces containing the desired amount of hydrophilicity and the desired amount of bioagent couplers. Hydrophilic spacer monomers (neutral, anionic and cationic) can be selected to produce appropriately charged hydrophilic spacers. Bioagent coupling monomers
10 can be selected based on pendant functional groups i.e., -OH, -NH₂, -CHO, -COOH, -NCO, needed or wanted from acrylic type monomers and is demonstrated in Fig. 23.

Graft copolymerization onto poly(ether urethane) with CeIV ion is the preferred medium. The monomers chosen to work with are acrylamide (AAM), acrylic acid (AA), 2-acrylamido-2-methylpropane sulfonic acid (AMPS), 2-hydroxyethyl methacrylate (HEMA) and N-(3-aminopropyl) methacrylamide hydrochloride (APMA).
15 These monomers were chosen based partly on their reactivity and water solubility characteristics. AAM, AA and AMPS monomers have been used as the biocompatible hydrophilic spacer monomers. HEMA and APMA monomers have been used as the bioagent coupling monomers. AAM has been used extensively as an affinity chromatographic matrix. AAM is a neutral hydrophilic monomer; therefore, polymers of AAM have shown little protein association. AMPS and AA are anionic hydrophilic monomers. Polymers of AMPS and copolymers of
20 AMPS and AA have been shown to have a heparin-like activity. HEMA is a hydroxyl containing monomer and APMA is an amino containing monomer.

Grafting can be engineered stoichiometrically to produce surfaces containing the desired amount of hydrophilicity and the desired amount of couplers. Hydrophilic spacer monomers (neutral, anionic and cationic) can be selected to produce appropriately charged hydrophilic spacers. Coupling monomers can be selected
25 based on pendant functional groups i.e., -OH, -NH₂, -CHO, -NCO needed or wanted for immobilizing heparin.

Either the AAM or AMPS monomer is used as the hydrophilic spacer monomer. The APMA monomer is used as the coupling monomer. AAM has been used extensively as an affinity chromatographic matrix. AAM is a neutral hydrophilic monomer, therefore, polymers of AAM have shown little protein association. APMA is an amino containing monomer, thus allowing the attachment of heparin.

Heparin is covalently attached to the amino groups on the APMA through a stabilized Schiff base reaction (reductive amination). The heparin is oxidized using either sodium periodate or nitrous acid. Both oxidation techniques form reactive aldehyde groups. These aldehyde groups react with the primary amines on APMA forming imines. The imines are stabilized using sodium cyanoborohydride.

The following Example 26 demonstrates a technique for heparinizing a polyurethane surface. Currently,
35 the technique yields polyurethane surfaces having approximately 5 times the heparin activity of Carmeda® coated polyurethane surfaces. Chandler loops prepared with the heparinized surface described have demonstrated that the coating prevented blood coagulation during a 2 hour study.

EXAMPLE 26

The heparin surface coating technique begins with the graft copolymerization of acrylamide (AAM) and N-(3-aminopropyl)methacrylamide (APMA) monomers onto a clean polyurethane surface with CeIV ion. The CeIV ion creates a free radical on the polyurethane surface which initiates the graft copolymerization of the acrylamides (Fig. 9a and 9b). The amount of surface amination (the graft copolymerization of APMA and AAM)
45 that takes place on the polyurethane surface can be measured via staining the surface with ponceau S dye, a negatively charged dye molecule. This dye ionically associates with primary amines on the aminated surface. After staining, the dye is released from the surface using SDS and quantified spectrophotometrically at 520 nm.

This aminated surface is then used for coupling heparin. The attachment of heparin consists of first oxidizing it with sodium m-periodate (NaIO₄) thus forming aldehyde groups (Fig. 10). These aldehyde groups are then covalently attached to primary amino groups in the grafted APMA/AAM through reductive amination (Schiff base reaction) (Fig. 11). Sodium cyanoborohydride (NaCNBH₃) is used to stabilize the imine linkages (Fig. 12).

The amount of heparin attached can be measured via staining the surface with toluidine blue dye, a positively charged dye that ionically associates with the negatively charged heparin. Toluidine blue is also released from the surface using SDS and is quantified spectrophotometrically at 640 nm. The heparin-activity of the surface is measured using a thrombin-ATIII activation assay. The assay begins by exposing heparin-derived samples to an excess of ATIII. Non-adsorbed ATIII is then removed by rapid rinsing in tris-buffered saline.

The amount of surface bound and activated ATIII is then estimated by incubating the samples with an excess of thrombin. After incubation, the residual thrombin is measured by reaction with a chromogenic substrate in a spectrophotometer. The change in absorbance at 405 nm is measured. See Figure 24 for the results.

5 EXAMPLE 27

The Cibacron Blue dye surface-derivatization technique begins with the graft polymerization of AMPS and HEMA monomers onto a polyurethane surface with $CeIV$ ion. The $CeIV$ ion creates a free radical on the polyurethane surface which initiates the graft copolymerization of the monomers. The HEMA monomer contains

10 a hydroxyl group that is then used for coupling Cibacron Blue dye.

Polyurethane surfaces containing covalently coupled Cibacron Blue dye were prepared by first placing the polyurethane samples into the following degassed grafting solution for 2 hours at ambient temperature.

40 g 2-acrylamido-2-methylpropane sulfonic acid (AMPS)

10 g 2-hydroxyethyl methacrylate (HEMA)

15 1.1 g ceric ammonium nitrate

1.3 g nitric acid

70 ml DI water

Following grafting, the samples were rinsed thoroughly in DI water. The AMPS/HEMA grafted samples were then placed into a Cibacron Blue dye solution consisting of 1.0 g Cibacron Blue dye, 4.0 g NaCl, 12.0 g

20 $NaHCO_3$ in 200 ml DI water overnight. The dyed samples were then thoroughly rinsed in DI water.

Claims

- 25 1. An article for use in contact with blood or blood products, said article having at least one exposed surface provided by a polymeric substrate with grafted thereto a graft polymer deriving from a hydrophilic vinyl monomer and with coupled to said graft polymer a bioactive agent, said graft polymer where non-ionically coupled to a said bioactive agent being a polymer or copolymer of a vinyl monomer selected from acrylic acid (AA), acrylamide (AAm), N-(3-aminopropyl)methacrylamide (APMA), 2-acrylamido-2-methylpropanesulfonic acid (AMPS) and salts thereof.
- 30 2. An article as claimed in claim 1 wherein said graft polymer is a hydrophilic polymer having pendant ionized or ionizable groups.
- 35 3. An article as claimed in either one of claims 1 and 2 wherein said graft polymer is a polymer or copolymer of a vinyl monomer selected from acrylic acid (AA), acrylamide (AAm), N-(3-aminopropyl)methacrylamide (APMA), 2-acrylamido-2-methylpropanesulfonic acid (AMPS) and salts thereof.
- 40 4. An article as claimed in claim 3 wherein said graft polymer is selected from AMPS polymers, APMA polymers, AMPS/hydroxyethylmethacrylate (HEMA) copolymers and APMA/AAm copolymers.
5. An article as claimed in any one of claims 1 to 4 wherein said bioactive agent is ionically coupled to said graft polymer.
- 45 6. An article as claimed in any one of claims 1 to 5 wherein said bioactive agent is an antibiotic or antimicrobial compound.
7. An article as claimed in claim 6 wherein said bioactive agent is gentamycin or silver ion.
8. An article as claimed in any one of claims 1 to 5 wherein said bioactive agent is basic fibroblast growth
- 50 factor.
9. An article as claimed in any one of claims 1 to 4 wherein said bioactive agent is covalently coupled to said graft polymer.
- 55 10. An article as claimed in any one of claims 1 to 5 and 9 wherein said bioactive agent is an albumin binding dye.
11. An article as claimed in claim 10 wherein said dye is selected from cibacron blue and blue dextran.

12. An article as claimed in any one of claims 1 to 4 and 9 wherein said bioactive agent is heparin.
13. An article as claimed in claim 1 wherein said graft polymer is an AMPS polymer and said bioactive agent is gentamycin.
- 5 14. An article as claimed in claim 1 wherein said graft polymer is an AMPS/HEMA or APMA/AAm copolymer and said bioactive agent is an albumin-binding dye coupled covalently thereto.
- 10 15. An article as claimed in claim 1 wherein said graft polymer is an APMA/AAm copolymer and said bioactive agent is heparin coupled covalently thereto.
16. An article as claimed in any one of claims 1 to 15 wherein said polymeric substrate is a polyurethane.
17. An article as claimed in claim 16 wherein said polymeric substrate is a polyetherurethane.
- 15 18. A process for the provision of an implantable article with a bioactive surface, said process comprising:
graft polymerizing on an exposed polymeric surface of said article a hydrophilic vinyl monomer having pendant groups optionally carrying an ionic charge,
if required chemically modifying the resultant graft polymer surface to provide an ionically charged or ionizable surface, and
20 coupling to said graft polymer surface a bioactive agent.
- 25 19. A method of preventing interaction between an article exposed to a body and a bioactive agent to be administered to the body wherein the agent has a (+) or (-) net ionic charge, which method comprises providing exposed parts of the article with a graft polymer surface which has a net ionic charge of the same polarity as that of said agent.

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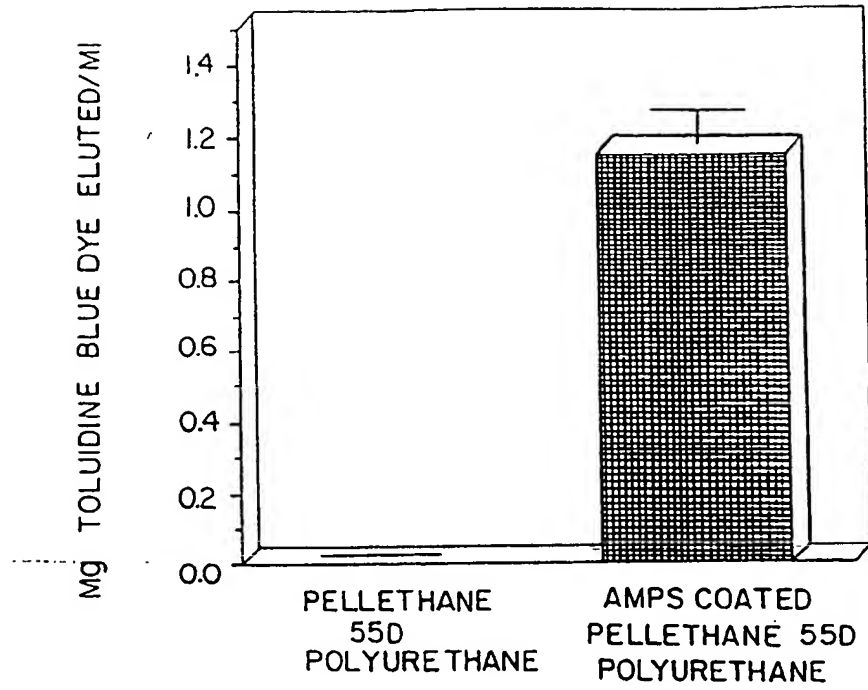


Fig.1

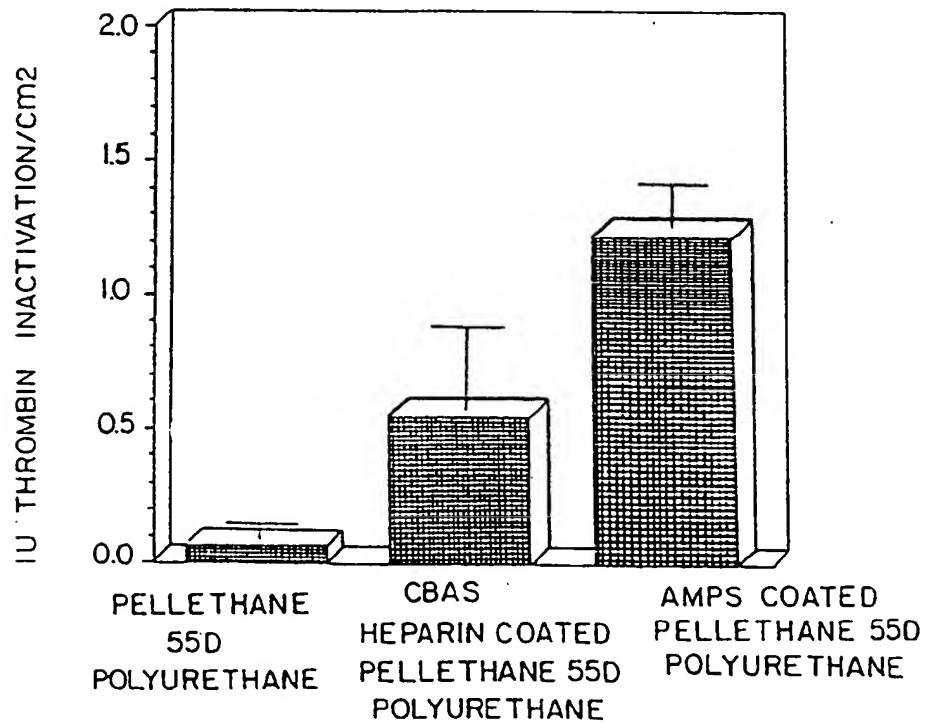
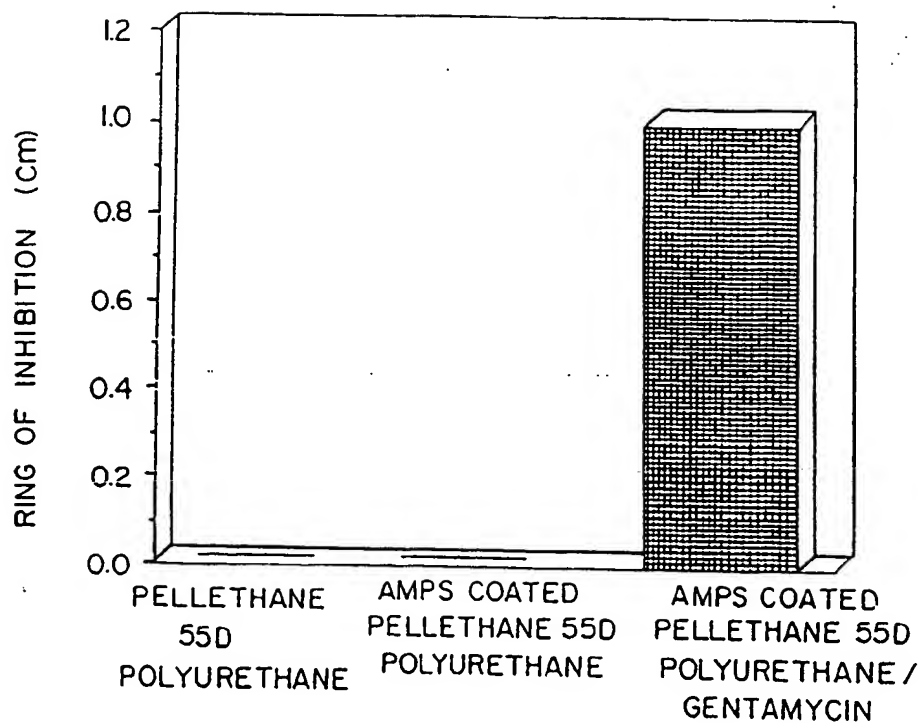
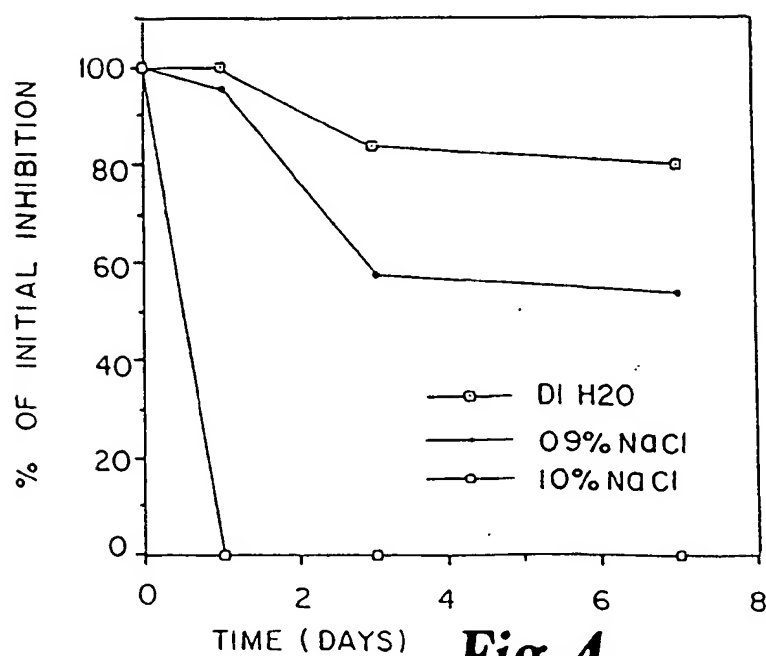


Fig.2

*Fig. 3**Fig. 4*

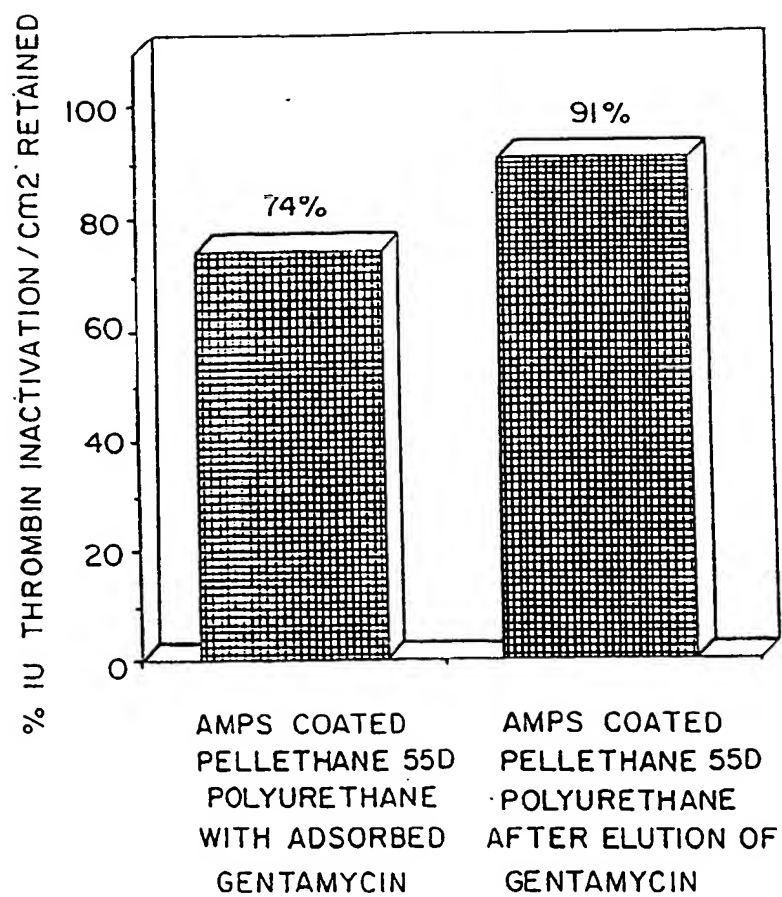
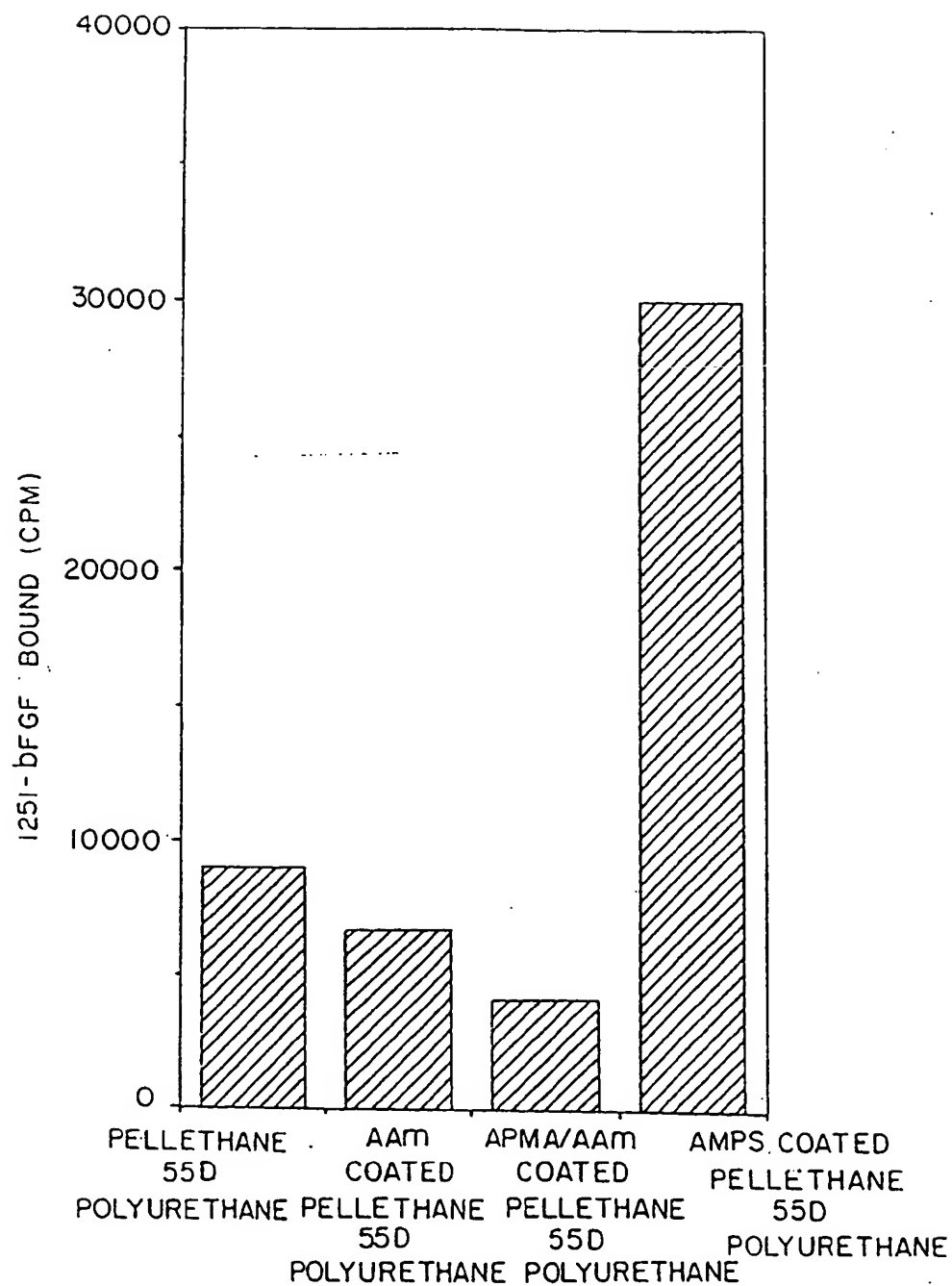
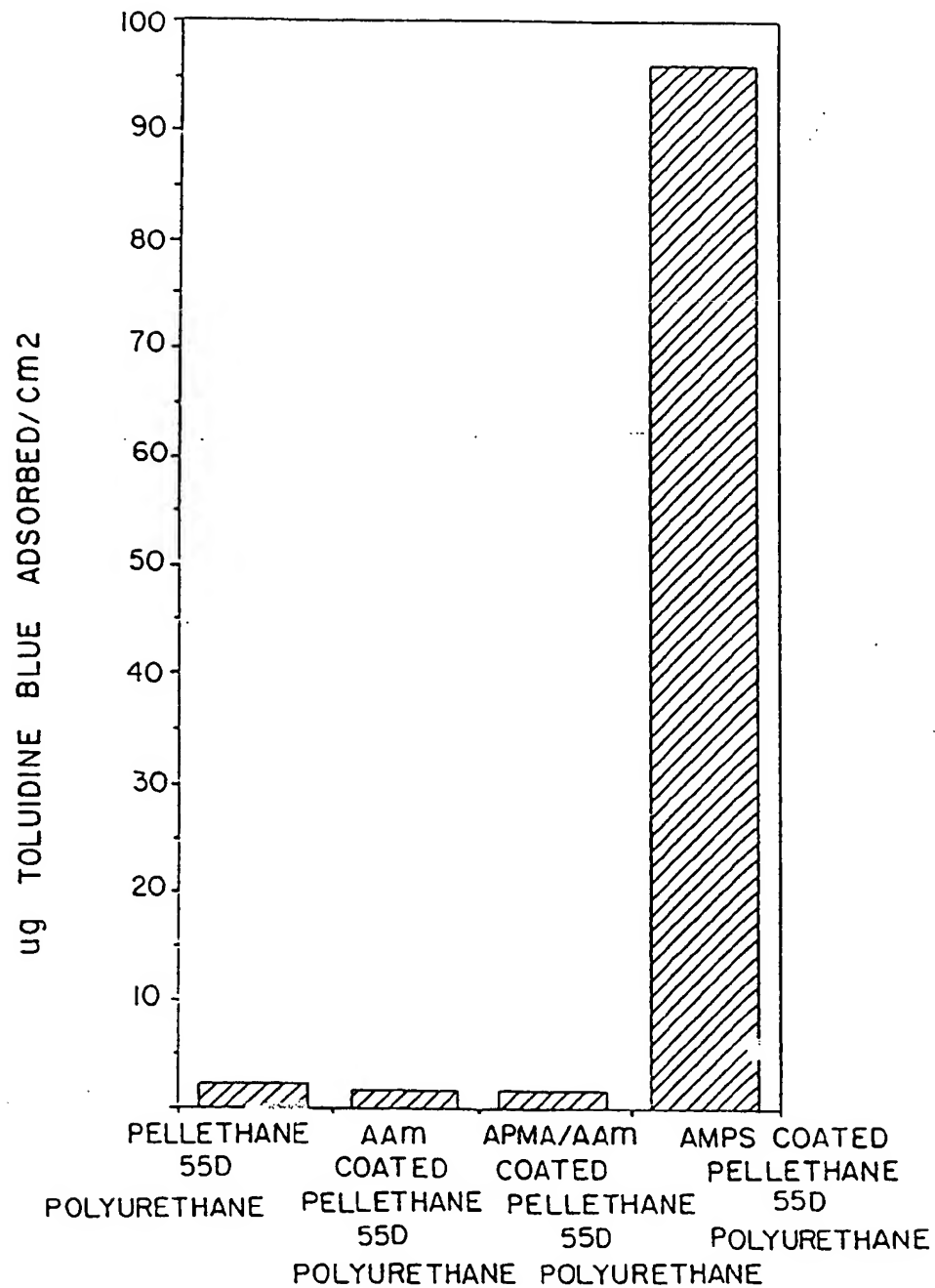


Fig. 5

**Fig. 6**

TOLUIDINE BLUE DYE ADSORPTION

*Fig. 7*

PONCEAU S DYE ADSORPTION

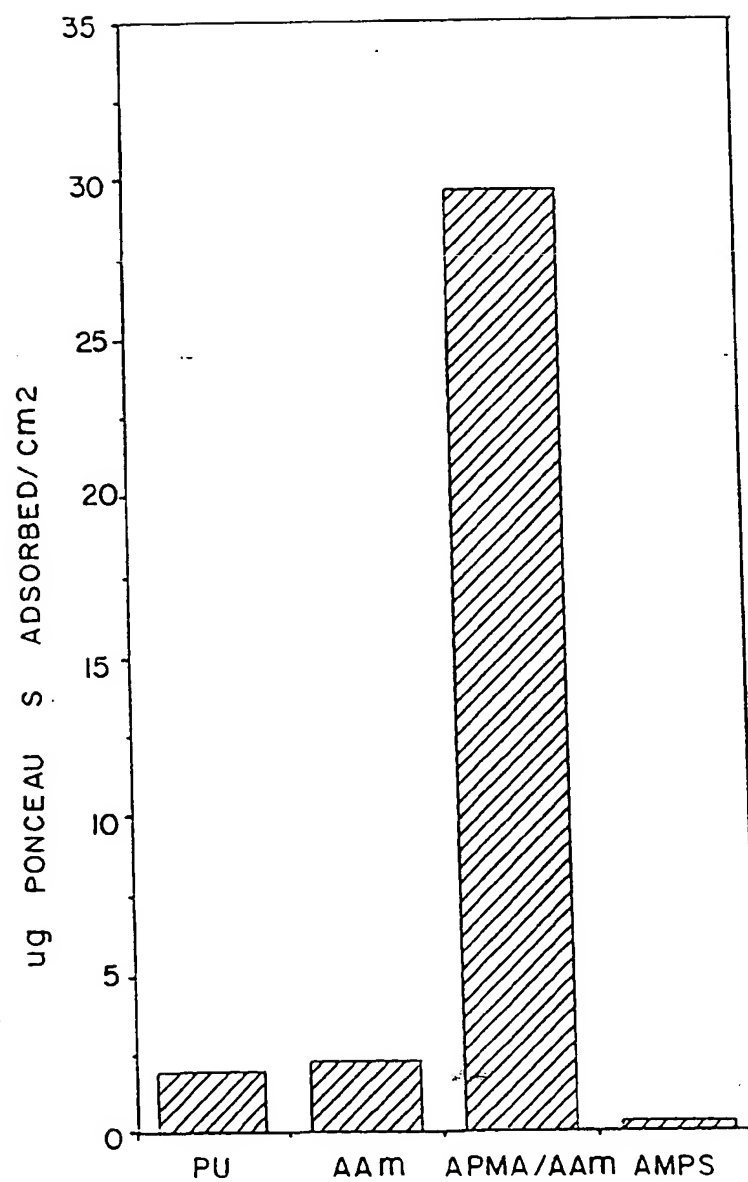
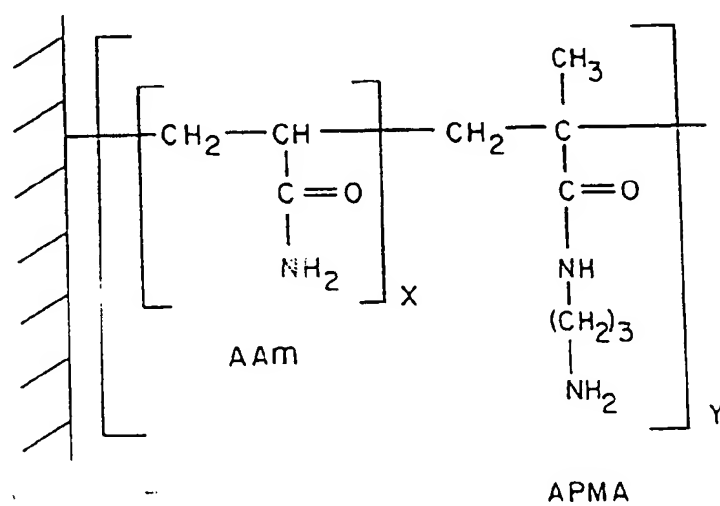
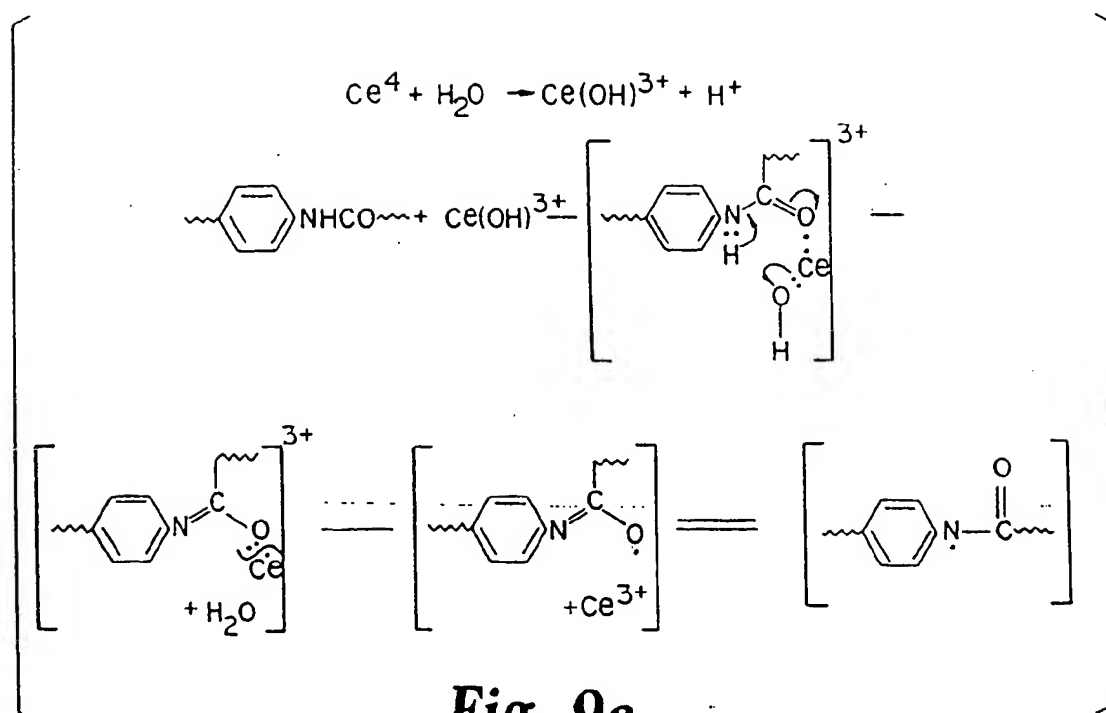


Fig.8

**Fig. 9b**

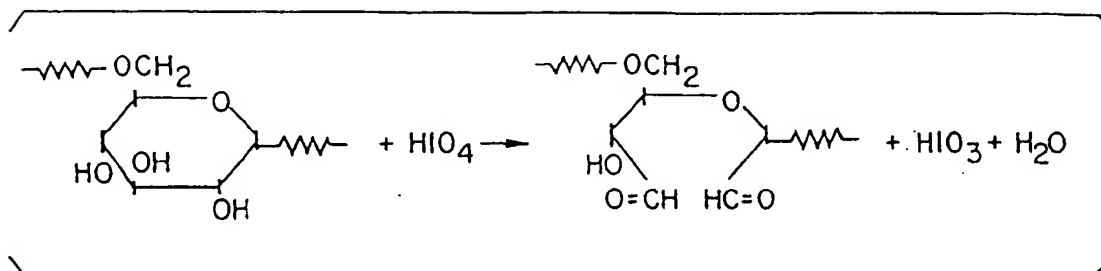


Fig.10

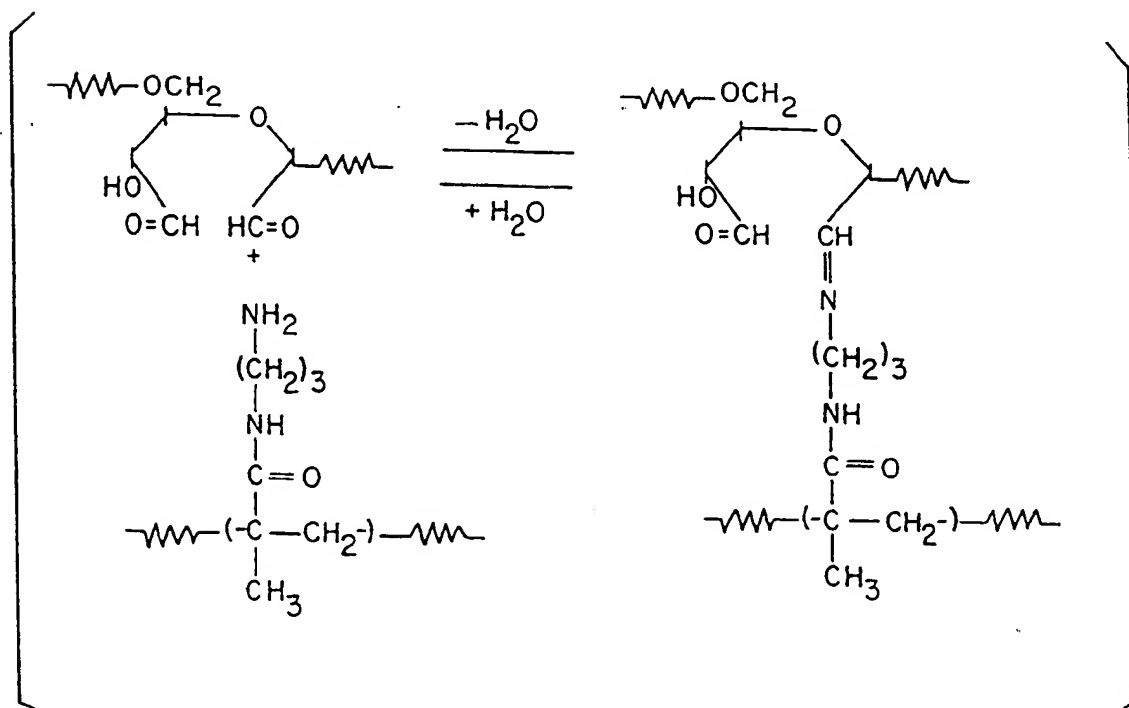
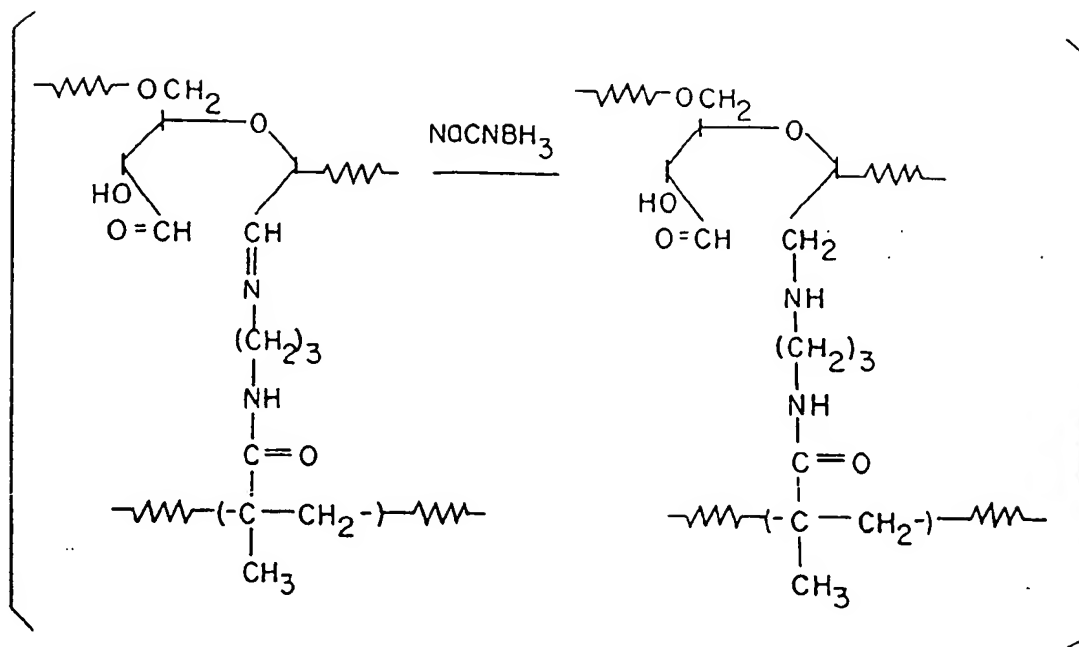
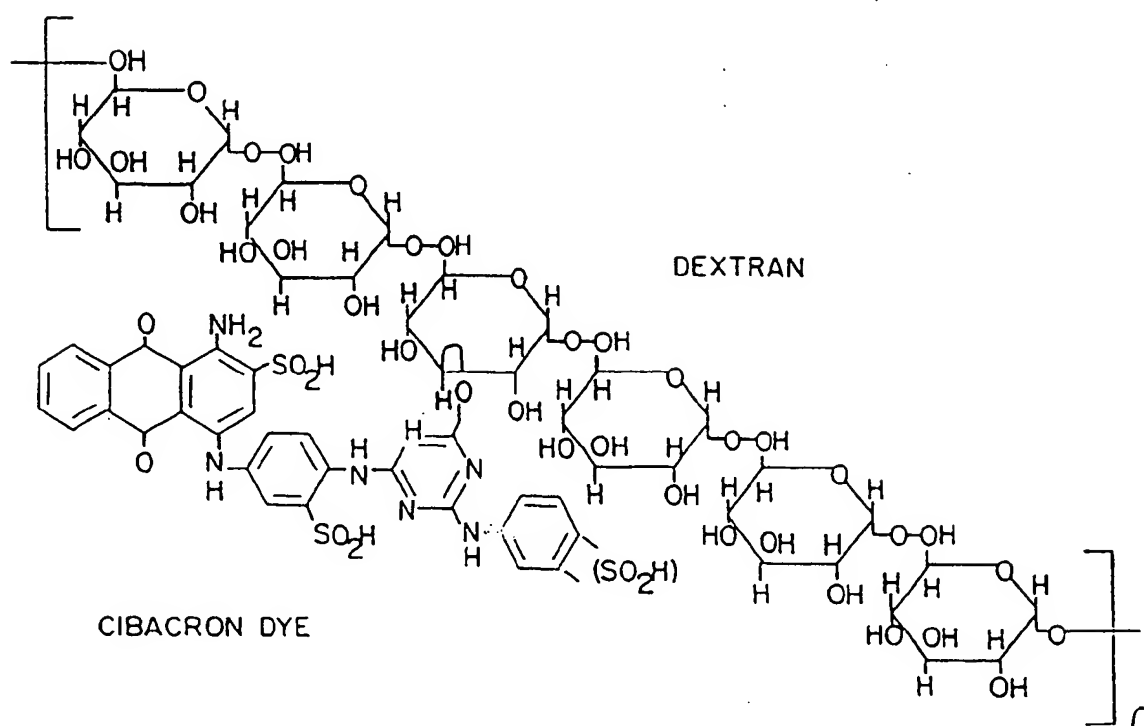
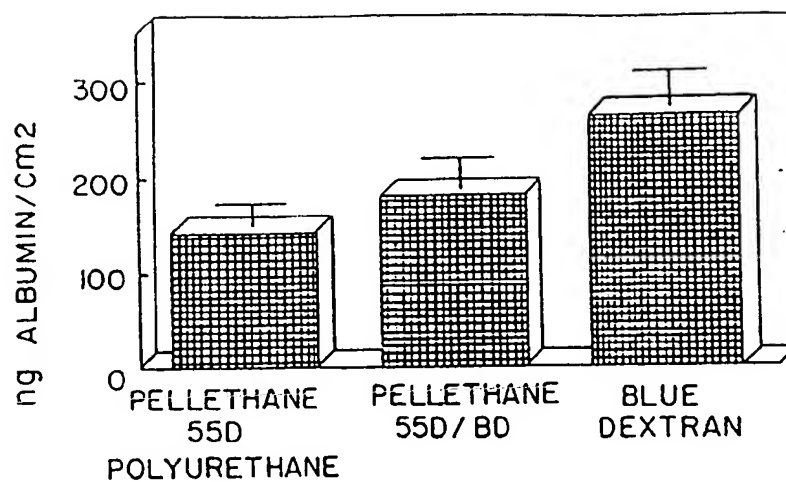
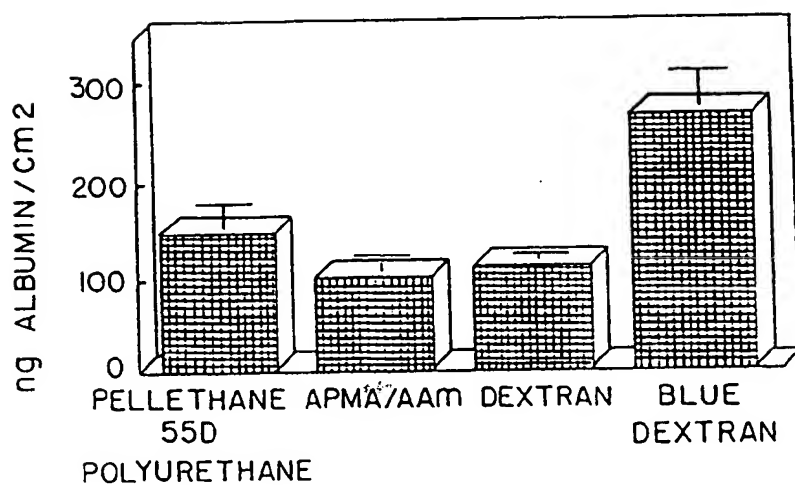


Fig. 11

**Fig. 12****Fig. 13**

*Fig. 14**Fig. 15*

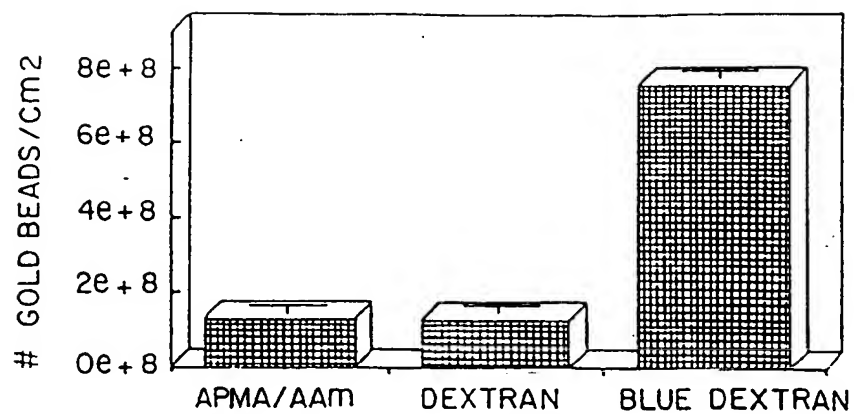


Fig.16

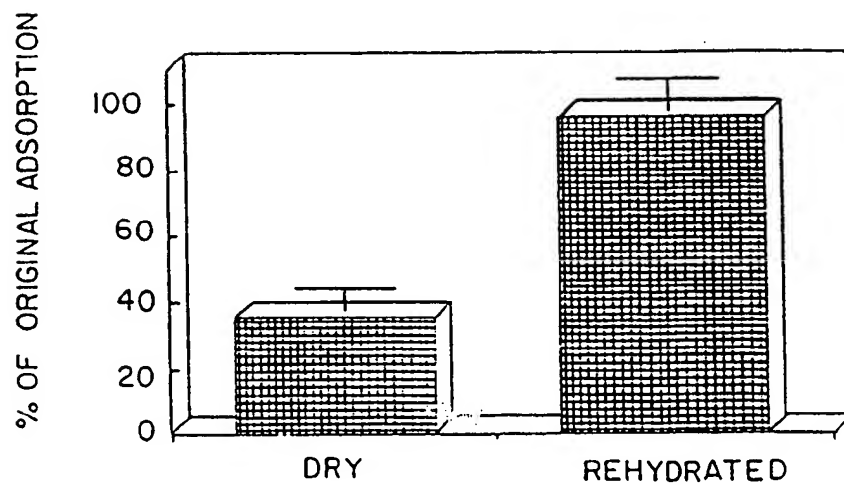


Fig.17

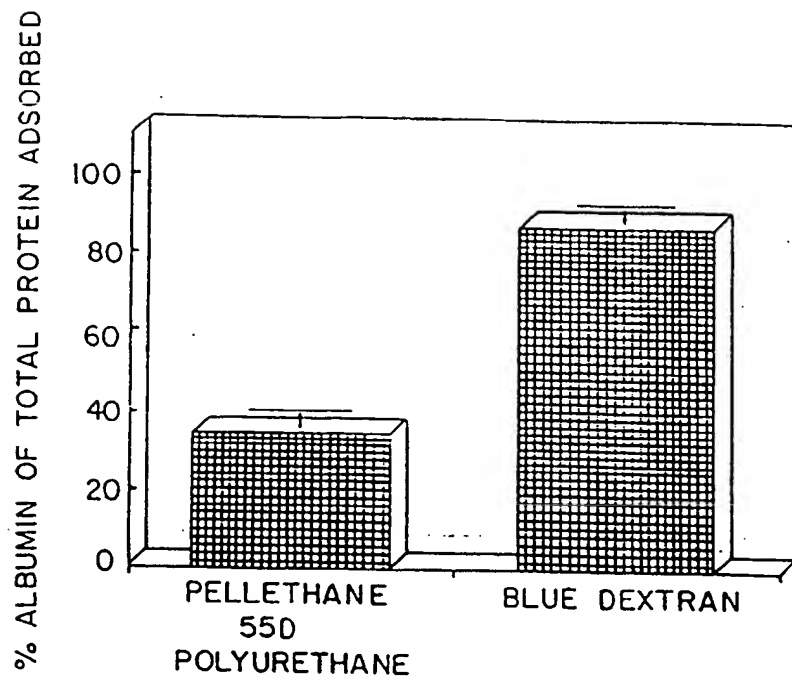


Fig.18

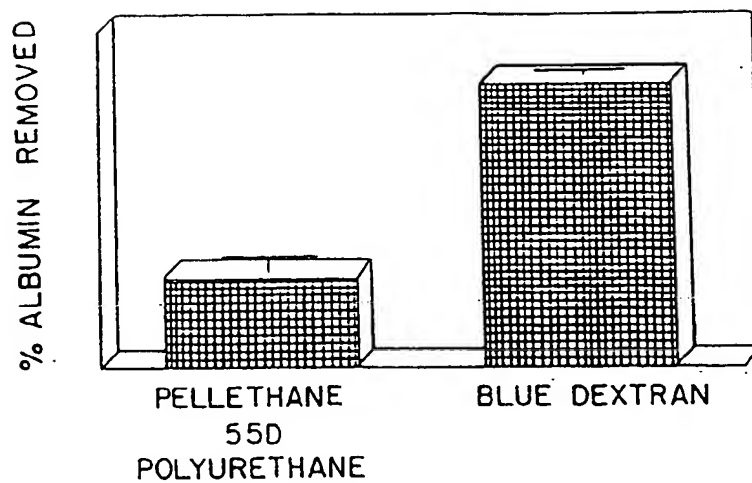


Fig. 19

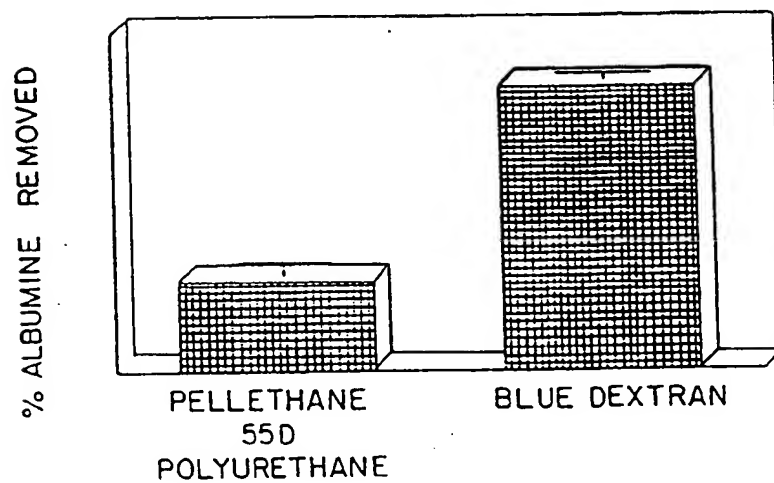


Fig. 20

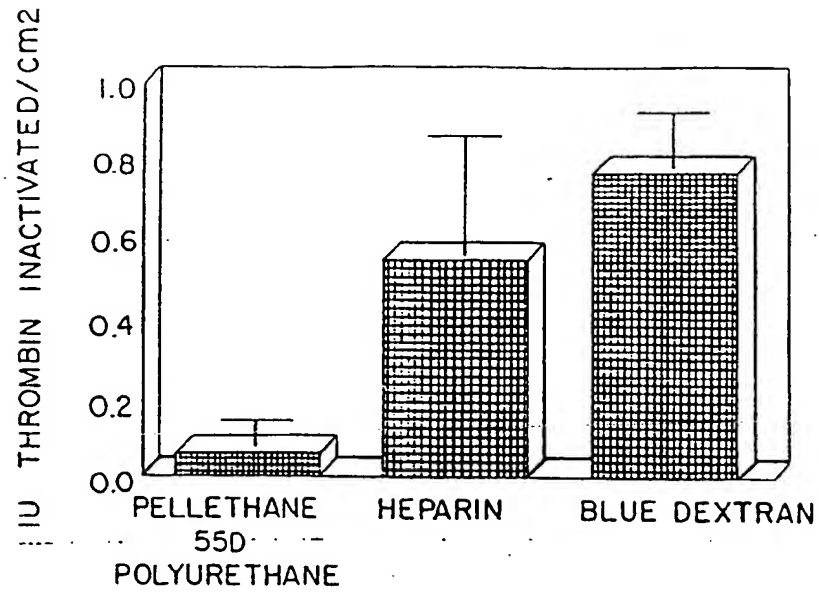


Fig. 21

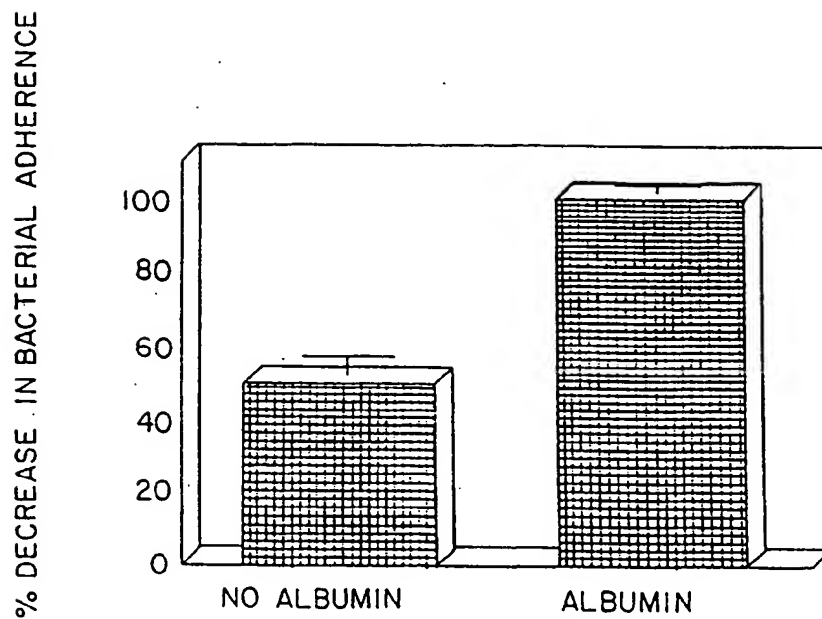
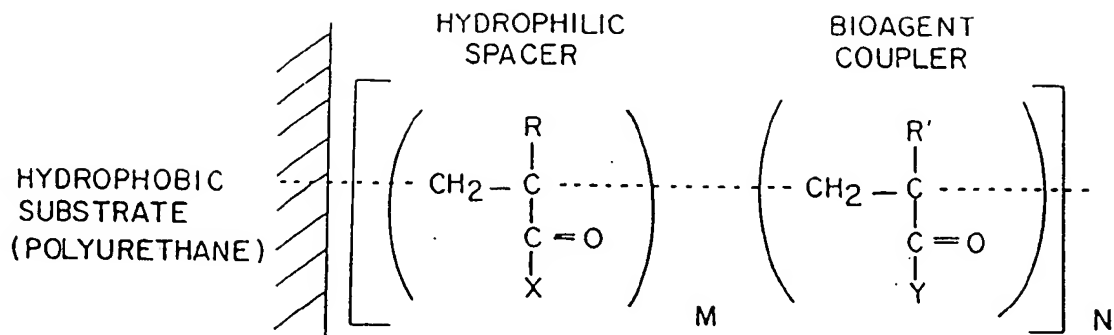


Fig. 22



- WHERE R IS EITHER H OR CH₃
- WHERE R' IS EITHER H OR CH₃
- WHERE X IS THE HYDROPHILIC SUBSTITUENT
- WHERE Y IS THE BIOAGENT COUPLING SUBSTITUENT
- WHERE M IS THE NUMBER OF BIOCOMPATIBLE HYDROPHILIC SPACER UNITS
- WHERE N IS THE NUMBER OF BIOCOMPATIBLE / BIOACTIVE UNITS

Fig. 23

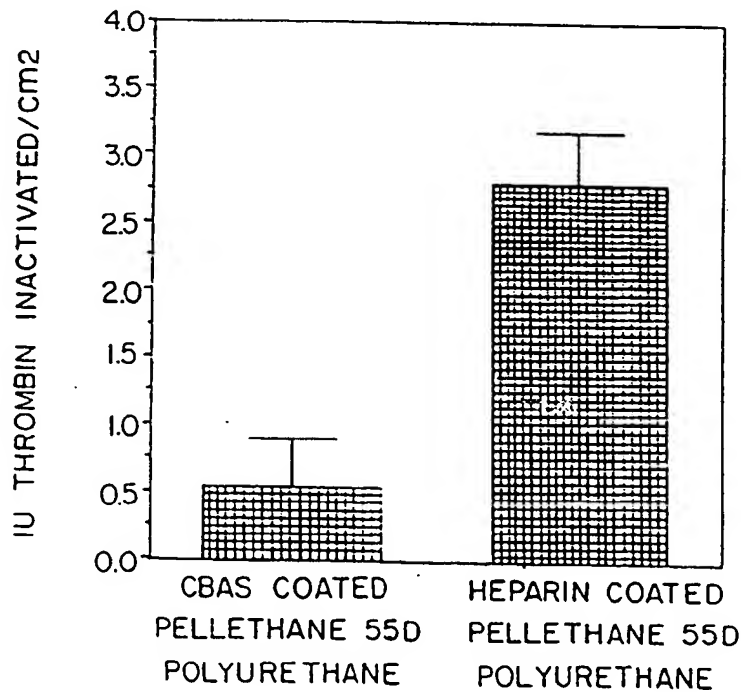


Fig. 24



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 93 30 8189

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cls)
P,X	US-A-5 229 172 (CAHALAN P.T.) * column 7, line 24 - line 66; claims 1,2,11-13 *	1-3, 5-10,12, 16-18	A61L27/00 A61L29/00 A61L33/00
P,X	EP-A-0 519 087 (HEWLETT-PACKARD) * page 5, line 35 - line 53 * * page 6, line 35 - line 39; claims 1,2,12,16 *	1,3,9,12	
X	WO-A-91 16932 (BIOCOMPATIBLES LIMITED) * claims 1,2,7,8 *	1,3,9,12	
X	US-A-5 080 924 (KAMEL I.) * column 8, line 56; claims 1,6-11 *	1,3,9,12	
X	EP-A-0 294 905 (SENTRON) * claims 6,15,16,23 *	1,3,9,12	
X	WO-A-87 00060 (BATELLE MEMORIAL INSTITUTE) * page 3, line 10 - line 28; claims 1-7 *	1,3,9,12	TECHNICAL FIELDS SEARCHED (Int.Cls.)
A	US-A-4 459 318 (HYANS T.E.) * claims 1,2 *	1,3	A61L
A	EP-A-0 470 443 (BECTON DICKINSON)		
A	WO-A-92 11877 (UNION CARBIDE)		
A	EP-A-0 184 465 (WARNER-LAMBERT)		
The present search report has been drawn up for all claims			
Place of search		Date of completion of the search	Examiner
THE HAGUE		3 March 1994	Peltre, C
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